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<p>(21) International Application Number: PCT/US94/03019 (22) International Filing Date: 18 March 1994 (18.03.94) (30) Priority Data: 08/033,923 19 March 1993 (19.03.93) US (60) Parent Application or Grant (63) Related by Continuation US 08/033,923 (CIP) Filed on 19 March 1993 (19.03.93) (71) Applicant (for all designated States except US): JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE [US/US]; 720 Rutland Avenue, Baltimore, MD 21205 (US). (72) Inventors; and (75) Inventors/Applicants (for US only): LEE, Se-Jin [US/US]; 6711 Chokeberry Road, Baltimore, MD 21209 (US). McPHERSON, Alexandra, C. [US/US]; 3905 Keswick Road, Baltimore, MD 21211 (US). (74) Agents: WETHERELL, John, R. et al.; Spensley Horn Jubas &amp; Lubitz, 1880 Century Park East, 5th Floor, Los Angeles, CA 90067 (US).</p>	<p>(81) Designated States: CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).  Published With international search report.</p>	
<p>(54) Title: GROWTH DIFFERENTIATION FACTOR-8</p> <p>(57) Abstract</p> <p>Growth differentiation factor-8 (GDF-8) is disclosed along with its polynucleotide sequence and amino acid sequence. Also disclosed are diagnostic and therapeutic methods of using the GDF-8 polypeptide and polynucleotide sequences.</p> <p style="text-align: right;">HEART LUNG THYMUS BRAIN KIDNEY SEMINAL VESICLE PANCREAS INTESTINE SPLEEN TESTIS MUSCLE LIVER OVARY FAT UTERUS</p> <p style="text-align: center;">- 2.9 kb</p>		

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## GROWTH DIFFERENTIATION FACTOR-8

This application is a continuation-in-part application of the U.S. Application Serial No. 08/033,923 filed on 3/19/93.

### BACKGROUND OF THE INVENTION

#### 5      1.      *Field of the Invention*

The invention relates generally to growth factors and specifically to a new member of the transforming growth factor beta (TGF- $\beta$ ) superfamily, which is denoted, growth differentiation factor-8 (GDF-8).

#### 2.      *Description of Related Art*

10      The transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily encompasses a group of structurally-related proteins which affect a wide range of differentiation processes during embryonic development. The family includes, Mullerian inhibiting substance (MIS), which is required for normal male sex development (Behringer, et al., Nature, 345:167, 1990), Drosophila decapentaplegic (DPP)  
15      gene product, which is required for dorsal-ventral axis formation and morphogenesis of the imaginal disks (Padgett, et al., Nature, 325:81-84, 1987), the Xenopus Vg-1 gene product, which localizes to the vegetal pole of eggs ((Weeks, et al., Cell, 51:861-867, 1987), the activins (Mason, et al., Biochem, Biophys. Res. Commun., 135:957-964, 1986), which can induce the formation  
20      of mesoderm and anterior structures in Xenopus embryos (Thomsen, et al., Cell, 63:485, 1990), and the bone morphogenetic proteins (BMPs, osteogenin, OP-1) which can induce de novo cartilage and bone formation (Sampath, et al., J. Biol. Chem., 265:13198, 1990). The TGF- $\beta$ s can influence a variety of differentiation processes, including adipogenesis, myogenesis, chondrogenesis,

hematopoiesis, and epithelial cell differentiation (for review, see Massague, *Cell* 49:437, 1987).

The proteins of the TGF- $\beta$  family are initially synthesized as a large precursor protein which subsequently undergoes proteolytic cleavage at a cluster of basic  
5 residues approximately 110-140 amino acids from the C-terminus. The C-terminal regions, or mature regions, of the proteins are all structurally related and the different family members can be classified into distinct subgroups based on the extent of their homology. Although the homologies within particular subgroups range from 70% to 90% amino acid sequence identity, the  
10 homologies between subgroups are significantly lower, generally ranging from only 20% to 50%. In each case, the active species appears to be a disulfide-linked dimer of C-terminal fragments. Studies have shown that when the pro-region of a member of the TGF- $\beta$  family is coexpressed with a mature region of another member of the TGF- $\beta$  family, intracellular dimerization and secretion of biologically active homodimers occur (Gray, A., and Maston, A., *Science*,  
15 247:1328, 1990). Additional studies by Hammonds, et al., (*Molec. Endocrin.* 5:149, 1991) showed that the use of the BMP-2 pro-region combined with the BMP-4 mature region led to dramatically improved expression of mature BMP-4. For most of the family members that have been studied, the homodimeric  
20 species has been found to be biologically active, but for other family members, like the inhibins (Ling, et al., *Nature*, 321:779, 1986) and the TGF- $\beta$ s (Cheifetz, et al., *Cell*, 48:409, 1987), heterodimers have also been detected, and these appear to have different biological properties than the respective homodimers.

Identification of new factors that are tissue-specific in their expression pattern  
25 will provide a greater understanding of that tissue's development and function.

**SUMMARY OF THE INVENTION**

The present invention provides a cell growth and differentiation factor, GDF-8, a polynucleotide sequence which encodes the factor, and antibodies which are immunoreactive with the factor. This factor appears to relate to various cell  
5 proliferative disorders, especially those involving those involving muscle, nerve, and adipose tissue.

Thus, in one embodiment, the invention provides a method for detecting a cell proliferative disorder of muscle, nerve, or fat origin and which is associated with GDF-8. In another embodiment, the invention provides a method for  
10 treating a cell proliferative disorder by suppressing or enhancing GDF-8 activity.

### BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 is a Northern blot showing expression of GDF-8 mRNA in adult tissues. The probe was a partial murine GDF-8 clone.

5 FIGURE 2 shows nucleotide and predicted amino acid sequences of murine GDF-8 (FIGURE 2a) and human GDF-8 (FIGURE 2b). The putative dibasic processing sites in the murine sequence are boxed.

FIGURE 3 shows the alignment of the C-terminal sequences of GDF-8 with other members of the TGF- $\beta$  superfamily. The conserved cysteine residues are boxed. Dashes denote gaps introduced in order to maximize alignment.

10 FIGURE 4 shows amino acid homologies among different members of the TGF- $\beta$  superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within particular subgroups.

15 FIGURE 5 shows the sequence of GDF-8. Nucleotide and amino acid sequences of murine (FIGURE 5a) and human (FIGURE 5b) GDF-8 cDNA clones are shown. Numbers indicate nucleotide position relative to the 5' end. Consensus N-linked glycosylation signals are shaded. The putative RXXR proteolytic cleavage sites are boxed.

20 FIGURE 6 shows a hydropathicity profile of GDF-8. Average hydrophobicity values for murine (FIGURE 6a) and human (FIGURE 6b) GDF-8 were calculated using the method of Kyte and Doolittle (J. Mol. Biol., 157:105-132, 1982). Positive numbers indicate increasing hydrophobicity.

FIGURE 7 shows a comparison of murine and human GDF-8 amino acid sequences. The predicted murine sequence is shown in the top lines and the predicted human sequence is shown in the bottom lines. Numbers indicate amino acid position relative to the N-terminus. Identities between the two sequences are denoted by a vertical line.

FIGURE 8 shows the expression of GDF-8 in bacteria. BL21 (DE3) (pLysS) cells carrying a pRSET/GDF-8 expression plasmid were induced with isopropylthio- $\beta$ -galactoside, and the GDF-8 fusion protein was purified by metal chelate chromatography. Lanes: total=total cell lysate; soluble=soluble protein fraction; insoluble=insoluble protein fraction (resuspended in 10 mM Tris pH 8.0, 50 mM sodium phosphate, 8 M urea, and 10 mM  $\beta$ -mercaptoethanol [buffer B]) loaded onto the column; pellet=insoluble protein fraction discarded before loading the column; flowthrough=proteins not bound by the column; washes=washes carried out in buffer B at the indicated pH's. Positions of molecular weight standards are shown at the right. Arrow indicates the position of the GDF-8 fusion protein.

FIGURE 9 shows the expression of GDF-8 in mammalian cells. Chinese hamster ovary cells were transfected with pMSXND/GDF-8 expression plasmids and selected in G418. Conditioned media from G418-resistant cells (prepared from cells transfected with constructs in which GDF-8 was cloned in either the antisense or sense orientation) were concentrated, electrophoresed under reducing conditions, blotted, and probed with anti-GDF-8 antibodies and [<sup>125</sup>I]iodoproteinA. Arrow indicates the position of the processed GDF-8 protein.

FIGURE 10 shows the expression of GDF-8 mRNA. Poly A-selected RNA (5  $\mu$ g each) prepared from adult tissues (FIGURE 10a) or placentas and embryos (FIGURE 10b) at the indicated days of gestation was electrophoresed on formaldehyde gels, blotted, and probed with full length murine GDF-8.

5      FIGURE 11 shows chromosomal mapping of human GDF-8. DNA samples prepared from human/rodent somatic cell hybrid lines were subjected to PCR, electrophoresed on agarose gels, blotted, and probed. The human chromosome contained in each of the hybrid cell lines is identified at the top of each of the first 24 lanes (1-22, X, and Y). In the lanes designated M, CHO,  
10     and H, the starting DNA template was total genomic DNA from mouse, hamster, and human sources, respectively. In the lane marked B1, no template DNA was used. Numbers at left indicate the mobilities of DNA standards.



## DETAILED DESCRIPTION OF THE INVENTION

The present invention provides a growth and differentiation factor, GDF-8 and a polynucleotide sequence encoding GDF-8. GDF-8 is expressed at highest levels in muscle and at lower levels in adipose tissue. In one embodiment, the invention provides a method for detection of a cell proliferative disorder of muscle, nerve, or fat origin which is associated with GDF-8 expression. In another embodiment, the invention provides a method for treating a cell proliferative disorder by using an agent which suppresses or enhances GDF-8 activity.

The TGF- $\beta$  superfamily consists of multifunctional polypeptides that control proliferation, differentiation, and other functions in many cell types. Many of the peptides have regulatory, both positive and negative, effects on other peptide growth factors. The structural homology between the GDF-8 protein of this invention and the members of the TGF- $\beta$  family, indicates that GDF-8 is a new member of the family of growth and differentiation factors. Based on the known activities of many of the other members, it can be expected that GDF-8 will also possess biological activities that will make it useful as a diagnostic and therapeutic reagent.

In particular, certain members of this superfamily have expression patterns or possess activities that relate to the function of the nervous system. For example, the inhibins and activins have been shown to be expressed in the brain (Meunier, et al., Proc. Natl. Acad. Sci., USA, 85:247, 1988; Sawchenko, et al., Nature, 334:615, 1988), and activin has been shown to be capable of functioning as a nerve cell survival molecule (Schubert, et al., Nature, 344:868, 1990). Another family member, namely, GDF-1, is nervous system-specific in its expression pattern (Lee, S.J., Proc. Natl. Acad. Sci., USA, 88:4250, 1991), and certain other family members, such as Vgr-1 (Lyons, et al., Proc. Natl

Acad. Sci., USA, 86:4554, 1989; Jones, et al., Development, 111:531, 1991), OP-1 (Ozkaynak, et al., J. Biol. Chem., 267:25220, 1992), and BMP-4 (Jones, et al., Development, 111:531, 1991), are also known to be expressed in the nervous system. Because it is known that skeletal muscle produces a factor or factors that promote the survival of motor neurons (Brown, Trends Neurosci., 7:10, 1984), the expression of GDF-8 in muscle suggests that one activity of GDF-8 may be as a trophic factor for neurons. In this regard, GDF-8 may have applications in the treatment of neurodegenerative diseases, such as amyotrophic lateral sclerosis, or in maintaining cells or tissues in culture prior to transplantation.

GDF-8 may also have applications in treating disease processes involving muscle, such as in musculodegenerative diseases or in tissue repair due to trauma. In this regard, many other members of the TGF- $\beta$  family are also important mediators of tissue repair. TGF- $\beta$  has been shown to have marked effects on the formation of collagen and to cause a striking angiogenic response in the newborn mouse (Roberts, et al., Proc. Natl. Acad. Sci., USA 83:4167, 1986). TGF- $\beta$  has also been shown to inhibit the differentiation of myoblasts in culture (Massague, et al., Proc. Natl. Acad. Sci., USA 83:8206, 1986). Moreover, because myoblast cells may be used as a vehicle for delivering genes to muscle for gene therapy, the properties of GDF-8 could be exploited for maintaining cells prior to transplantation or for enhancing the efficiency of the fusion process.

The expression of GDF-8 in adipose tissue also raises the possibility of applications for GDF-8 in the treatment of obesity or of disorders related to abnormal proliferation of adipocytes. In this regard, TGF- $\beta$  has been shown to be a potent inhibitor of adipocyte differentiation in vitro (Ignotz and Massague, Proc. Natl. Acad. Sci., USA 82:8530, 1985).

The term "substantially pure" as used herein refers to GDF-8 which is substantially free of other proteins, lipids, carbohydrates or other materials with which it is naturally associated. One skilled in the art can purify GDF-8 using standard techniques for protein purification. The substantially pure polypeptide will yield a single major band on a non-reducing polyacrylamide gel. The purity of the GDF-8 polypeptide can also be determined by amino-terminal amino acid sequence analysis. GDF-8 polypeptide includes functional fragments of the polypeptide, as long as the activity of GDF-8 remains. Smaller peptides containing the biological activity of GDF-8 are included in the invention.

The invention provides polynucleotides encoding the GDF-8 protein. These polynucleotides include DNA, cDNA and RNA sequences which encode GDF-8. It is understood that all polynucleotides encoding all or a portion of GDF-8 are also included herein, as long as they encode a polypeptide with GDF-8 activity. Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, GDF-8 polynucleotide may be subjected to site-directed mutagenesis. The polynucleotide sequence for GDF-8 also includes antisense sequences. The polynucleotides of the invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included in the invention as long as the amino acid sequence of GDF-8 polypeptide encoded by the nucleotide sequence is functionally unchanged.

Specifically disclosed herein is a genomic DNA sequence containing a portion of the GDF-8 gene. The sequence contains an open reading frame corresponding to the predicted C-terminal region of the GDF-8 precursor protein. The encoded polypeptide is predicted to contain two potential proteolytic processing sites (KR and RR). Cleavage of the precursor at the downstream site would generate a mature biologically active C-terminal

fragment of 109 amino acids with a predicted molecular weight of approximately 12,400. Also, disclosed are full length murine and human GDF-8 cDNA sequences. The murine pre-pro-GDF-8 protein is 376 amino acids in length, which is encoded by a 2676 base pair nucleotide sequence, beginning  
5 at nucleotide 104 and extending to a TGA stop codon at nucleotide 1232. The human GDF-8 protein is 375 amino acids and is encoded by a 2743 base pair sequence, with the open reading frame beginning at nucleotide 59 and extending to nucleotide 1184.

The C-terminal region of GDF-8 following the putative proteolytic processing  
10 site shows significant homology to the known members of the TGF- $\beta$  superfamily. The GDF-8 sequence contains most of the residues that are highly conserved in other family members (see FIGURE 3). Like the TGF- $\beta$ s and inhibin  $\beta$ s, GDF-8 contains an extra pair of cysteine residues in addition to the 7 cysteines found in virtually all other family members. Among the known  
15 family members, GDF-8 is most homologous to Vgr-1 (45% sequence identity) (see FIGURE 4).

Minor modifications of the recombinant GDF-8 primary amino acid sequence may result in proteins which have substantially equivalent activity as compared to the GDF-8 polypeptide described herein. Such modifications may be  
20 deliberate, as by site-directed mutagenesis, or may be spontaneous. All of the polypeptides produced by these modifications are included herein as long as the biological activity of GDF-8 still exists. Further, deletion of one or more amino acids can also result in a modification of the structure of the resultant molecule without significantly altering its biological activity. This can lead to the  
25 development of a smaller active molecule which would have broader utility. For example, one can remove amino or carboxy terminal amino acids which are not required for GDF-8 biological activity.

The nucleotide sequence encoding the GDF-8 polypeptide of the invention includes the disclosed sequence and conservative variations thereof. The term "conservative variation" as used herein denotes the replacement of an amino acid residue by another, biologically similar residue. Examples of conservative variations include the substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine, and the like. The term "conservative variation" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid provided that antibodies raised to the substituted polypeptide also immunoreact with the unsubstituted polypeptide.

DNA sequences of the invention can be obtained by several methods. For example, the DNA can be isolated using hybridization techniques which are well known in the art. These include, but are not limited to: 1) hybridization of genomic or cDNA libraries with probes to detect homologous nucleotide sequences, 2) polymerase chain reaction (PCR) on genomic DNA or cDNA using primers capable of annealing to the DNA sequence of interest, and 3) antibody screening of expression libraries to detect cloned DNA fragments with shared structural features.

Preferably the GDF-8 polynucleotide of the invention is derived from a mammalian organism, and most preferably from a mouse, rat, or human. Screening procedures which rely on nucleic acid hybridization make it possible to isolate any gene sequence from any organism, provided the appropriate probe is available. Oligonucleotide probes, which correspond to a part of the sequence encoding the protein in question, can be synthesized chemically. This requires that short, oligopeptide stretches of amino acid sequence must be known. The DNA sequence encoding the protein can be deduced from the

genetic code, however, the degeneracy of the code must be taken into account. It is possible to perform a mixed addition reaction when the sequence is degenerate. This includes a heterogeneous mixture of denatured double-stranded DNA. For such screening, hybridization is preferably performed on either single-stranded DNA or denatured double-stranded DNA. Hybridization is particularly useful in the detection of cDNA clones derived from sources where an extremely low amount of mRNA sequences relating to the polypeptide of interest are present. In other words, by using stringent hybridization conditions directed to avoid non-specific binding, it is possible, for example, to allow the autoradiographic visualization of a specific cDNA clone by the hybridization of the target DNA to that single probe in the mixture which is its complete complement (Wallace, et al., Nucl. Acid Res., 9:879, 1981).

The development of specific DNA sequences encoding GDF-8 can also be obtained by: 1) isolation of double-stranded DNA sequences from the genomic DNA; 2) chemical manufacture of a DNA sequence to provide the necessary codons for the polypeptide of interest; and 3) in vitro synthesis of a double-stranded DNA sequence by reverse transcription of mRNA isolated from a eukaryotic donor cell. In the latter case, a double-stranded DNA complement of mRNA is eventually formed which is generally referred to as cDNA.

Of the three above-noted methods for developing specific DNA sequences for use in recombinant procedures, the isolation of genomic DNA isolates is the least common. This is especially true when it is desirable to obtain the microbial expression of mammalian polypeptides due to the presence of introns.

The synthesis of DNA sequences is frequently the method of choice when the entire sequence of amino acid residues of the desired polypeptide product is known. When the entire sequence of amino acid residues of the desired polypeptide is not known, the direct synthesis of DNA sequences is not possible and the method of choice is the synthesis of cDNA sequences. Among the standard procedures for isolating cDNA sequences of interest is the formation of plasmid- or phage-carrying cDNA libraries which are derived from reverse transcription of mRNA which is abundant in donor cells that have a high level of genetic expression. When used in combination with polymerase chain reaction technology, even rare expression products can be cloned. In those cases where significant portions of the amino acid sequence of the polypeptide are known, the production of labeled single or double-stranded DNA or RNA probe sequences duplicating a sequence putatively present in the target cDNA may be employed in DNA/DNA hybridization procedures which are carried out on cloned copies of the cDNA which have been denatured into a single-stranded form (Jay, et al., Nucl. Acid Res., 11:2325, 1983).

A cDNA expression library, such as lambda gt11, can be screened indirectly for GDF-8 peptides having at least one epitope, using antibodies specific for GDF-8. Such antibodies can be either polyclonally or monoclonally derived and used to detect expression product indicative of the presence of GDF-8 cDNA.

DNA sequences encoding GDF-8 can be expressed in vitro by DNA transfer into a suitable host cell. "Host cells" are cells in which a vector can be propagated and its DNA expressed. The term also includes any progeny of the subject host cell. It is understood that all progeny may not be identical to the parental cell since there may be mutations that occur during replication. However, such progeny are included when the term "host cell" is used.

Methods of stable transfer, meaning that the foreign DNA is continuously maintained in the host, are known in the art.

In the present invention, the GDF-8 polynucleotide sequences may be inserted into a recombinant expression vector. The term "recombinant expression  
5 vector" refers to a plasmid, virus or other vehicle known in the art that has been manipulated by insertion or incorporation of the GDF-8 genetic sequences. Such expression vectors contain a promoter sequence which facilitates the efficient transcription of the inserted genetic sequence of the host. The expression vector typically contains an origin of replication, a promoter, as  
10 well as specific genes which allow phenotypic selection of the transformed cells. Vectors suitable for use in the present invention include, but are not limited to the T7-based expression vector for expression in bacteria (Rosenberg, et al., *Gene*, 56:125, 1987), the pMSXND expression vector for expression in mammalian cells (Lee and Nathans, *J. Biol. Chem.*, 263:3521,  
15 1988) and baculovirus-derived vectors for expression in insect cells. The DNA segment can be present in the vector operably linked to regulatory elements, for example, a promoter (e.g., T7, metallothionein I, or polyhedrin promoters).

Polynucleotide sequences encoding GDF-8 can be expressed in either  
20 prokaryotes or eukaryotes. Hosts can include microbial, yeast, insect and mammalian organisms. Methods of expressing DNA sequences having eukaryotic or viral sequences in prokaryotes are well known in the art. Biologically functional viral and plasmid DNA vectors capable of expression and replication in a host are known in the art. Such vectors are used to incorporate DNA sequences of the invention. Preferably, the mature C-terminal  
25 region of GDF-8 is expressed from a cDNA clone containing the entire coding sequence of GDF-8. Alternatively, the C-terminal portion of GDF-8 can be expressed as a fusion protein with the pro- region of another member of the TGF- $\beta$  family or co-expressed with another pro- region (see for example,



Hammonds, et al., *Molec. Endocrin.* 5:149, 1991; Gray, A., and Mason, A., *Science*, 247:1328, 1990).

Transformation of a host cell with recombinant DNA may be carried out by conventional techniques as are well known to those skilled in the art. Where  
5 the host is prokaryotic, such as *E. coli*, competent cells which are capable of DNA uptake can be prepared from cells harvested after exponential growth phase and subsequently treated by the  $\text{CaCl}_2$  method using procedures well known in the art. Alternatively,  $\text{MgCl}_2$  or  $\text{RbCl}$  can be used. Transformation can also be performed after forming a protoplast of the host cell if desired.

10 When the host is a eukaryote, such methods of transfection of DNA as calcium phosphate co-precipitates, conventional mechanical procedures such as microinjection, electroporation, insertion of a plasmid encased in liposomes, or virus vectors may be used. Eukaryotic cells can also be cotransformed with DNA sequences encoding the GDF-8 of the invention, and a second foreign  
15 DNA molecule encoding a selectable phenotype, such as the herpes simplex thymidine kinase gene. Another method is to use a eukaryotic viral vector, such as simian virus 40 (SV40) or bovine papilloma virus, to transiently infect or transform eukaryotic cells and express the protein. (see for example, *Eukaryotic Viral Vectors*, Cold Spring Harbor Laboratory, Gluzman ed., 1982).

20 Isolation and purification of microbial expressed polypeptide, or fragments thereof, provided by the invention, may be carried out by conventional means including preparative chromatography and immunological separations involving monoclonal or polyclonal antibodies.

The invention includes antibodies immunoreactive with GDF-8 polypeptide or  
25 functional fragments thereof. Antibody which consists essentially of pooled monoclonal antibodies with different epitopic specificities, as well as distinct

monoclonal antibody preparations are provided. Monoclonal antibodies are made from antigen containing fragments of the protein by methods well known to those skilled in the art (Kohler, et al., Nature, 256:495, 1975). The term antibody as used in this invention is meant to include intact molecules as well  
5 as fragments thereof, such as Fab and F(ab')<sub>2</sub>, which are capable of binding an epitopic determinant on GDF-8.

The term "cell-proliferative disorder" denotes malignant as well as non-malignant cell populations which often appear to differ from the surrounding tissue both morphologically and genotypically. Malignant cells (i.e. cancer) develop as a  
10 result of a multistep process. The GDF-8 polynucleotide that is an antisense molecule is useful in treating malignancies of the various organ systems, particularly, for example, cells in muscle or adipose tissue. Essentially, any disorder which is etiologically linked to altered expression of GDF-8 could be considered susceptible to treatment with a GDF-8 suppressing reagent. One  
15 such disorder is a malignant cell proliferative disorder, for example.

The invention provides a method for detecting a cell proliferative disorder of muscle or adipose tissue which comprises contacting an anti-GDF-8 antibody with a cell suspected of having a GDF-8 associated disorder and detecting binding to the antibody. The antibody reactive with GDF-8 is labeled with a  
20 compound which allows detection of binding to GDF-8. For purposes of the invention, an antibody specific for GDF-8 polypeptide may be used to detect the level of GDF-8 in biological fluids and tissues. Any specimen containing a detectable amount of antigen can be used. A preferred sample of this invention is muscle tissue. The level of GDF-8 in the suspect cell can be  
25 compared with the level in a normal cell to determine whether the subject has a GDF-8-associated cell proliferative disorder. Preferably the subject is human.

The antibodies of the invention can be used in any subject in which it is desirable to administer in vitro or in vivo immunodiagnosis or immunotherapy. The antibodies of the invention are suited for use, for example, in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. In addition, the antibodies in these immunoassays can be detectably labeled in various ways. Examples of types of immunoassays which can utilize antibodies of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA) and the sandwich (immunometric) assay. Detection of the antigens using the antibodies of the invention can be done utilizing immunoassays which are run in either the forward, reverse, or simultaneous modes, including immunohistochemical assays on physiological samples. Those of skill in the art will know, or can readily discern, other immunoassay formats without undue experimentation.

The antibodies of the invention can be bound to many different carriers and used to detect the presence of an antigen comprising the polypeptide of the invention. Examples of well-known carriers include glass, polystyrene, polypropylene, polyethylene, dextran, nylon, amylases, natural and modified celluloses, polyacrylamides, agaroses and magnetite. The nature of the carrier can be either soluble or insoluble for purposes of the invention. Those skilled in the art will know of other suitable carriers for binding antibodies, or will be able to ascertain such, using routine experimentation.

There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, fluorescent compounds, colloidal metals, chemiluminescent compounds, phosphorescent compounds, and bioluminescent compounds. Those of ordinary skill in the art will know of

other suitable labels for binding to the antibody, or will be able to ascertain such, using routine experimentation.

Another technique which may also result in greater sensitivity consists of coupling the antibodies to low molecular weight haptens. These haptens can  
5 then be specifically detected by means of a second reaction. For example, it is common to use such haptens as biotin, which reacts with avidin, or dinitrophenyl, puridoxal, and fluorescein, which can react with specific anti-hapten antibodies.

10 In using the monoclonal antibodies of the invention for the in vivo detection of antigen, the detectably labeled antibody is given a dose which is diagnostically effective. The term "diagnostically effective" means that the amount of detectably labeled monoclonal antibody is administered in sufficient quantity to enable detection of the site having the antigen comprising a polypeptide of the invention for which the monoclonal antibodies are specific.

15 The concentration of detectably labeled monoclonal antibody which is administered should be sufficient such that the binding to those cells having the polypeptide is detectable compared to the background. Further, it is desirable that the detectably labeled monoclonal antibody be rapidly cleared from the circulatory system in order to give the best target-to-background  
20 signal ratio.

As a rule, the dosage of detectably labeled monoclonal antibody for in vivo diagnosis will vary depending on such factors as age, sex, and extent of disease of the individual. Such dosages may vary, for example, depending on whether multiple injections are given, antigenic burden, and other factors  
25 known to those of skill in the art.

For in vivo diagnostic imaging, the type of detection instrument available is a major factor in selecting a given radioisotope. The radioisotope chosen must have a type of decay which is detectable for a given type of instrument. Still another important factor in selecting a radioisotope for in vivo diagnosis is that deleterious radiation with respect to the host is minimized. Ideally, a radioisotope used for in vivo imaging will lack a particle emission, but produce a large number of photons in the 140-250 keV range, which may readily be detected by conventional gamma cameras.

For in vivo diagnosis radioisotopes may be bound to immunoglobulin either directly or indirectly by using an intermediate functional group. Intermediate functional groups which often are used to bind radioisotopes which exist as metallic ions to immunoglobulins are the bifunctional chelating agents such as diethylenetriaminepentaacetic acid (DTPA) and ethylenediaminetetraacetic acid (EDTA) and similar molecules. Typical examples of metallic ions which can be bound to the monoclonal antibodies of the invention are  $^{111}\text{In}$ ,  $^{97}\text{Ru}$ ,  $^{67}\text{Ga}$ ,  $^{68}\text{Ga}$ ,  $^{72}\text{As}$ ,  $^{89}\text{Zr}$ , and  $^{201}\text{Tl}$ .

The monoclonal antibodies of the invention can also be labeled with a paramagnetic isotope for purposes of in vivo diagnosis, as in magnetic resonance imaging (MRI) or electron spin resonance (ESR). In general, any conventional method for visualizing diagnostic imaging can be utilized. Usually gamma and positron emitting radioisotopes are used for camera imaging and paramagnetic isotopes for MRI. Elements which are particularly useful in such techniques include  $^{157}\text{Gd}$ ,  $^{55}\text{Mn}$ ,  $^{162}\text{Dy}$ ,  $^{52}\text{Cr}$ , and  $^{56}\text{Fe}$ .

The monoclonal antibodies of the invention can be used in vitro and in vivo to monitor the course of amelioration of a GDF-8-associated disease in a subject. Thus, for example, by measuring the increase or decrease in the number of cells expressing antigen comprising a polypeptide of the invention or changes

in the concentration of such antigen present in various body fluids, it would be possible to determine whether a particular therapeutic regimen aimed at ameliorating the GDF-8-associated disease is effective. The term "ameliorate" denotes a lessening of the detrimental effect of the GDF-8-associated disease  
5 in the subject receiving therapy.

The present invention identifies a nucleotide sequence that can be expressed in an altered manner as compared to expression in a normal cell, therefore it is possible to design appropriate therapeutic or diagnostic techniques directed to this sequence. Thus, where a cell-proliferative disorder is associated with  
10 the expression of GDF-8, nucleic acid sequences that interfere with GDF-8 expression at the translational level can be used. This approach utilizes, for example, antisense nucleic acid and ribozymes to block translation of a specific GDF-8 mRNA, either by masking that mRNA with an antisense nucleic acid or by cleaving it with a ribozyme. Such disorders include neurodegenerative  
15 diseases, for example.

Antisense nucleic acids are DNA or RNA molecules that are complementary to at least a portion of a specific mRNA molecule (Weintraub, *Scientific American*, 262:40, 1990). In the cell, the antisense nucleic acids hybridize to the corresponding mRNA, forming a double-stranded molecule. The antisense  
20 nucleic acids interfere with the translation of the mRNA, since the cell will not translate a mRNA that is double-stranded. Antisense oligomers of about 15 nucleotides are preferred, since they are easily synthesized and are less likely to cause problems than larger molecules when introduced into the target GDF-8-producing cell. The use of antisense methods to inhibit the in vitro  
25 translation of genes is well known in the art (Marcus-Sakura, *Anal.Biochem.*, 172:289, 1988).

Ribozymes are RNA molecules possessing the ability to specifically cleave other single-stranded RNA in a manner analogous to DNA restriction endonucleases. Through the modification of nucleotide sequences which encode these RNAs, it is possible to engineer molecules that recognize specific nucleotide sequences in an RNA molecule and cleave it (Cech, J.Amer.Med. Assn., 260:3030, 1988). A major advantage of this approach is that, because they are sequence-specific, only mRNAs with particular sequences are inactivated.

There are two basic types of ribozymes namely, tetrahymena-type (Hasselhoff, Nature, 334:585, 1988) and "hammerhead"-type. Tetrahymena-type ribozymes recognize sequences which are four bases in length, while "hammerhead"-type ribozymes recognize base sequences 11-18 bases in length. The longer the recognition sequence, the greater the likelihood that the sequence will occur exclusively in the target mRNA species. Consequently, hammerhead-type ribozymes are preferable to tetrahymena-type ribozymes for inactivating a specific mRNA species and 18-based recognition sequences are preferable to shorter recognition sequences.

The present invention also provides gene therapy for the treatment of cell proliferative or immunologic disorders which are mediated by GDF-8 protein. Such therapy would achieve its therapeutic effect by introduction of the GDF-8 antisense polynucleotide into cells having the proliferative disorder. Delivery of antisense GDF-8 polynucleotide can be achieved using a recombinant expression vector such as a chimeric virus or a colloidal dispersion system. Especially preferred for therapeutic delivery of antisense sequences is the use of targeted liposomes.

Various viral vectors which can be utilized for gene therapy as taught herein include adenovirus, herpes virus, vaccinia, or, preferably, an RNA virus such as a retrovirus. Preferably, the retroviral vector is a derivative of a murine or avian retrovirus. Examples of retroviral vectors in which a single foreign gene can be inserted include, but are not limited to: Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), and Rous Sarcoma Virus (RSV). A number of additional retroviral vectors can incorporate multiple genes. All of these vectors can transfer or incorporate a gene for a selectable marker so that transduced cells can be identified and generated. By inserting a GDF-8 sequence of interest into the viral vector, along with another gene which encodes the ligand for a receptor on a specific target cell, for example, the vector is now target specific. Retroviral vectors can be made target specific by attaching, for example, a sugar, a glycolipid, or a protein. Preferred targeting is accomplished by using an antibody to target the retroviral vector. Those of skill in the art will know of, or can readily ascertain without undue experimentation, specific polynucleotide sequences which can be inserted into the retroviral genome or attached to a viral envelope to allow target specific delivery of the retroviral vector containing the GDF-8 antisense polynucleotide.

Since recombinant retroviruses are defective, they require assistance in order to produce infectious vector particles. This assistance can be provided, for example, by using helper cell lines that contain plasmids encoding all of the structural genes of the retrovirus under the control of regulatory sequences within the LTR. These plasmids are missing a nucleotide sequence which enables the packaging mechanism to recognize an RNA transcript for encapsidation. Helper cell lines which have deletions of the packaging signal include, but are not limited to  $\psi$ 2, PA317 and PA12, for example. These cell lines produce empty virions, since no genome is packaged. If a retroviral vector is introduced into such cells in which the packaging signal is intact, but



the structural genes are replaced by other genes of interest, the vector can be packaged and vector virion produced.

Alternatively, NIH 3T3 or other tissue culture cells can be directly transfected with plasmids encoding the retroviral structural genes gag, pol and env, by  
5 conventional calcium phosphate transfection. These cells are then transfected with the vector plasmid containing the genes of interest. The resulting cells release the retroviral vector into the culture medium.

Another targeted delivery system for GDF-8 antisense polynucleotides is a colloidal dispersion system. Colloidal dispersion systems include macromole-  
10 cule complexes, nanocapsules, microspheres, beads, and lipid-based systems including oil-in-water emulsions, micelles, mixed micelles, and liposomes. The preferred colloidal system of this invention is a liposome. Liposomes are artificial membrane vesicles which are useful as delivery vehicles in vitro and in vivo. It has been shown that large unilamellar vesicles (LUV), which range  
15 in size from 0.2-4.0  $\mu\text{m}$  can encapsulate a substantial percentage of an aqueous buffer containing large macromolecules. RNA, DNA and intact virions can be encapsulated within the aqueous interior and be delivered to cells in a biologically active form (Fraley, et al., Trends Biochem. Sci., 6:77, 1981). In addition to mammalian cells, liposomes have been used for delivery of  
20 polynucleotides in plant, yeast and bacterial cells. In order for a liposome to be an efficient gene transfer vehicle, the following characteristics should be present: (1) encapsulation of the genes of interest at high efficiency while not compromising their biological activity; (2) preferential and substantial binding to a target cell in comparison to non-target cells; (3) delivery of the aqueous  
25 contents of the vesicle to the target cell cytoplasm at high efficiency; and (4) accurate and effective expression of genetic information (Mannino, et al., Biotechniques, 6:682, 1988).

The composition of the liposome is usually a combination of phospholipids, particularly high-phase-transition-temperature phospholipids, usually in combination with steroids, especially cholesterol. Other phospholipids or other lipids may also be used. The physical characteristics of liposomes depend on  
5 pH, ionic strength, and the presence of divalent cations.

Examples of lipids useful in liposome production include phosphatidyl compounds, such as phosphatidylglycerol, phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, sphingolipids, cerebroside, and gangliosides. Particularly useful are diacylphosphatidylglycerols, where the lipid  
10 moiety contains from 14-18 carbon atoms, particularly from 16-18 carbon atoms, and is saturated. Illustrative phospholipids include egg phosphatidylcholine, dipalmitoylphosphatidylcholine and distearoylphosphatidylcholine.

The targeting of liposomes can be classified based on anatomical and mechanistic factors. Anatomical classification is based on the level of  
15 selectivity, for example, organ-specific, cell-specific, and organelle-specific. Mechanistic targeting can be distinguished based upon whether it is passive or active. Passive targeting utilizes the natural tendency of liposomes to distribute to cells of the reticulo-endothelial system (RES) in organs which contain sinusoidal capillaries. Active targeting, on the other hand, involves  
20 alteration of the liposome by coupling the liposome to a specific ligand such as a monoclonal antibody, sugar, glycolipid, or protein, or by changing the composition or size of the liposome in order to achieve targeting to organs and cell types other than the naturally occurring sites of localization.

The surface of the targeted delivery system may be modified in a variety of  
25 ways. In the case of a liposomal targeted delivery system, lipid groups can be incorporated into the lipid bilayer of the liposome in order to maintain the

targeting ligand in stable association with the liposomal bilayer. Various linking groups can be used for joining the lipid chains to the targeting ligand.

5 Due to the expression of GDF-8 in muscle and adipose tissue, there are a variety of applications using the polypeptide, polynucleotide, and antibodies of the invention, related to these tissues. Such applications include treatment of cell proliferative disorders involving these and other tissues, such as neural tissue. In addition, GDF-8 may be useful in various gene therapy procedures.

10 The data in Example 6 shows that the human GDF-8 gene is located on chromosome 2. By comparing the chromosomal location of GDF-8 with the map positions of various human disorders, it should be possible to determine whether mutations in the GDF-8 gene are involved in the etiology of human diseases. For example, an autosomal recessive form of juvenile amyotrophic lateral sclerosis has been shown to map to chromosome 2 (Hentati, et al., Neurology, 42 [Suppl.3]:201, 1992). More precise mapping of GDF-8 and analysis of DNA from these patients may indicate that GDF-8 is, in fact, the gene affected in this disease. In addition, GDF-8 is useful for distinguishing chromosome 2 from other chromosomes.

20 The following examples are intended to illustrate but not limit the invention. While they are typical of those that might be used, other procedures known to those skilled in the art may alternatively be used.

**EXAMPLE 1**  
**IDENTIFICATION AND ISOLATION OF A NOVEL**  
**TGF- $\beta$  FAMILY MEMBER**

To identify a new member of the TGF- $\beta$  superfamily, degenerate  
5 oligonucleotides were designed which corresponded to two conserved regions  
among the known family members: one region spanning the two tryptophan  
residues conserved in all family members except MIS and the other region  
spanning the invariant cysteine residues near the C-terminus. These primers  
were used for polymerase chain reactions on mouse genomic DNA followed  
10 by subcloning the PCR products using restriction sites placed at the 5' ends  
of the primers, picking individual E. coli colonies carrying these subcloned  
inserts, and using a combination of random sequencing and hybridization  
analysis to eliminate known members of the superfamily.

GDF-8 was identified from a mixture of PCR products obtained with the primers  
15 SJL141: 5'-CCGGAATTCGGITGG(G/C/A)A(G/A/T/C)(A/G)A(T/C)TGG(A/G)TI  
(A/G)TI(T/G)CICC-3' (SEQ ID NO:1)  
SJL147: 5'-CCGGAATTC(G/A)CAI(G/C)C(G/A)CA(G/A)CT(G/A/T/C)  
TCIACI(G/A)(T/C)CAT-3' (SEQ ID NO:2)

PCR using these primers was carried out with 2  $\mu$ g mouse genomic DNA at  
20 94°C for 1 min, 50°C for 2 min, and 72°C for 2 min for 40 cycles.

PCR products of approximately 280 bp were gel-purified, digested with Eco RI,  
gel-purified again, and subcloned in the Bluescript vector (Stratagene, San  
Diego, CA). Bacterial colonies carrying individual subclones were picked into  
96 well microtiter plates, and multiple replicas were prepared by plating the  
25 cells onto nitrocellulose. The replicate filters were hybridized to probes

representing known members of the family, and DNA was prepared from non-hybridizing colonies for sequence analysis.

The primer combination of SJL141 and SJL147, encoding the amino acid sequences GW(H/Q/N/K/D/E)(D/N)W(V/I/M)(V/I/M)(A/S)P (SEQ ID NO:9) and  
5 M(V/I/M/T/A)V(D/E)SC(G/A)C (SEQ ID NO:10), respectively, yielded four previously identified sequences (BMP-4, inhibin  $\beta$ B, GDF-3 and GDF-5) and one novel sequence, which was designated GDF-8, among 110 subclones analyzed.

Human GDF-8 was isolated using the primers:

10 ACM13: 5'-CGCGGATCCAGAAGTCAAGGTGACAGACACAC-3' (SEQ ID NO:3);  
and  
ACM14: 5'-CGCGGATCCTCCTCATGAGCACCCACAGCGGTC-3' (SEQ ID NO:4)

15 PCR using these primers was carried out with one  $\mu$ g human genomic DNA at 94°C for 1 min, 58°C for 2 min, and 72°C for 2 min for 30 cycles. The PCR product was digested with Bam HI, gel-purified, and subcloned in the Bluescript vector (Stratagene, San Francisco, CA).

## EXAMPLE 2

### EXPRESSION PATTERN AND SEQUENCE OF GDF-8

20 To determine the expression pattern of GDF-8, RNA samples prepared from a variety of adult tissues were screened by Northern analysis. RNA isolation and Northern analysis were carried out as described previously (Lee, S.-J., Mol. Endocrinol., 4:1034, 1990) except that hybridization was carried out in 5X SSPE, 10% dextran sulfate, 50% formamide, 1% SDS, 200  $\mu$ g/ml salmon DNA, and 0.1% each of bovine serum albumin, ficoll, and polyvinylpyrrolidone. Five

micrograms of twice poly A-selected RNA prepared from each tissue (except for muscle, for which only 2  $\mu$ g RNA was used) were electrophoresed on formaldehyde gels, blotted, and probed with GDF-8. As shown in FIGURE 1, the GDF-8 probe detected a single mRNA species expressed at highest levels in muscle and at significantly lower levels in adipose tissue.

To obtain a larger segment of the GDF-8 gene, a mouse genomic library was screened with a probe derived from the GDF-8 PCR product. The partial sequence of a GDF-8 genomic clone is shown in FIGURE 2a. The sequence contains an open reading frame corresponding to the predicted C-terminal region of the GDF-8 precursor protein. The predicted GDF-8 sequence contains two potential proteolytic processing sites, which are boxed. Cleavage of the precursor at the second of these sites would generate a mature C-terminal fragment 109 amino acids in length with a predicted molecular weight of 12,400. The partial sequence of human GDF-8 is shown in FIGURE 2b. Assuming no PCR-induced errors during the isolation of the human clone, the human and mouse amino acid sequences in this region are 100% identical.

The C-terminal region of GDF-8 following the putative proteolytic processing site shows significant homology to the known members of the TGF- $\beta$  superfamily (FIGURE 3). FIGURE 3 shows the alignment of the C-terminal sequences of GDF-8 with the corresponding regions of human GDF-1 (Lee, Proc. Natl. Acad. Sci. USA, 88:4250-4254, 1991), human BMP-2 and 4 (Wozney, et al., Science, 242:1528-1534, 1988), human Vgr-1 (Celeste, et al., Proc. Natl. Acad. Sci. USA, 87:9843-9847, 1990), human OP-1 (Ozkaynak, et al., EMBO J., 9:2085-2093, 1990), human BMP-5 (Celeste, et al., Proc. Natl. Acad. Sci. USA, 87:9843-9847, 1990), human BMP-3 (Wozney, et al., Science, 242:1528-1534, 1988), human MIS (Cate, et al., Cell, 45:685-698, 1986), human inhibin alpha,  $\beta$ A, and  $\beta$ B (Mason, et al., Biochem, Biophys. Res. Commun., 135:957-964, 1986), human TGF- $\beta$ 1 (Derynck, et al., Nature, 316:701-705,

1985), human TGF- $\beta$ 2 (deMartin, et al., EMBO J., 6:3673-3677, 1987), and human TGF- $\beta$ 3 (ten Dijke, et al., Proc. Natl. Acad. Sci. USA, 85:4715-4719, 1988). The conserved cysteine residues are boxed. Dashes denote gaps introduced in order to maximize the alignment.

5 GDF-8 contains most of the residues that are highly conserved in other family members, including the seven cysteine residues with their characteristic spacing. Like the TGF- $\beta$ s and inhibin  $\beta$ s, GDF-8 also contains two additional cysteine residues. In the case of TGF- $\beta$ 2, these two additional cysteine residues are known to form an intramolecular disulfide bond (Daopin, et al.,  
10 Science, 257:369, 1992; Schlunegger and Grutter, Nature, 358:430, 1992).

FIGURE 4 shows the amino acid homologies among the different members of the TGF- $\beta$  superfamily. Numbers represent percent amino acid identities between each pair calculated from the first conserved cysteine to the C-terminus. Boxes represent homologies among highly-related members within  
15 particular subgroups. In this region, GDF-8 is most homologous to Vgr-1 (45% sequence identity).

### EXAMPLE 3

#### ISOLATION OF cDNA CLONES ENCODING MURINE AND HUMAN GDF-8

In order to isolate full-length cDNA clones encoding murine and human GDF-8,  
20 cDNA libraries were prepared in the lambda ZAP II vector (Stratagene) using RNA prepared from skeletal muscle. From 5  $\mu$ g of twice poly A-selected RNA prepared from murine and human muscle, cDNA libraries consisting of 4.4 million and 1.9 million recombinant phage, respectively, were constructed according to the instructions provided by Stratagene. These libraries were  
25 screened without amplification. Library screening and characterization of cDNA

inserts were carried out as described previously (Lee, Mol. Endocrinol, 4:1034-1040).

From  $2.4 \times 10^6$  recombinant phage screened from the murine muscle cDNA library, greater than 280 positive phage were identified using a murine GDF-8 probe derived from a genomic clone, as described in Example 1. The entire nucleotide sequence of the longest cDNA insert analyzed is shown in FIGURE 5a and SEQ ID NO:11. The 2676 base pair sequence contains a single long open reading frame beginning with a methionine codon at nucleotide 104 and extending to a TGA stop codon at nucleotide 1232. Upstream of the putative initiating methionine codon is an in-frame stop codon at nucleotide 23. The predicted pre-pro-GDF-8 protein is 376 amino acids in length. The sequence contains a core of hydrophobic amino acids at the N-terminus suggestive of a signal peptide for secretion (FIGURE 6a), one potential N-glycosylation site at asparagine 72, a putative RXXR proteolytic cleavage site at amino acids 264-267, and a C-terminal region showing significant homology to the known members of the TGF- $\beta$  superfamily. Cleavage of the precursor protein at the putative RXXR site would generate a mature C-terminal GDF-8 fragment 109 amino acids in length with a predicted molecular weight of approximately 12,400.

From  $1.9 \times 10^6$  recombinant phage screened from the human muscle cDNA library, 4 positive phage were identified using a human GDF-8 probe derived by polymerase chain reaction on human genomic DNA. The entire nucleotide sequence of the longest cDNA insert is shown in FIGURE 5b and SEQ ID NO:13. The 2743 base pair sequence contains a single long open reading frame beginning with a methionine codon at nucleotide 59 and extending to a TGA stop codon at nucleotide 1184. The predicted pre-pro-GDF-8 protein is 375 amino acids in length. The sequence contains a core of hydrophobic amino acids at the N-terminus suggestive of a signal peptide for secretion



(FIGURE 6b), one potential N-glycosylation site at asparagine 71, and a putative RXXR proteolytic cleavage site at amino acids 263-266. FIGURE 7 shows a comparison of the predicted murine (top) and human (bottom) GDF-8 amino acid sequences. Numbers indicate amino acid position relative to the N-terminus. Identities between the two sequences are denoted by a vertical line. Murine and human GDF-8 are approximately 94% identical in the predicted pro-regions and 100% identical following the predicted RXXR cleavage sites.

**EXAMPLE 4**  
**PREPARATION OF ANTIBODIES AGAINST GDF-8 AND**  
**EXPRESSION OF GDF-8 IN MAMMALIAN CELLS**

In order to prepare antibodies against GDF-8, GDF-8 antigen was expressed  
5 as a fusion protein in bacteria. A portion of murine GDF-8 cDNA spanning  
amino acids 268-376 (mature region) was inserted into the pRSET vector  
(Invitrogen) such that the GDF-8 coding sequence was placed in frame with the  
initiating methionine codon present in the vector; the resulting construct  
created an open reading frame encoding a fusion protein with a molecular  
10 weight of approximately 16,600. The fusion construct was transformed into  
BL21 (DE3) (pLysS) cells, and expression of the fusion protein was induced by  
treatment with isopropylthio- $\beta$ -galactoside as described (Rosenberg, et al.,  
Gene, 56:125-135). The fusion protein was then purified by metal chelate  
chromatography according to the instructions provided by Invitrogen. A  
15 Coomassie blue-stained gel of unpurified and purified fusion proteins is shown  
in FIGURE 8.

The purified fusion protein was used to immunize both rabbits and chickens.  
Immunization of rabbits was carried out by Spring Valley Labs (Sykesville, MD),  
and immunization of chickens was carried out by HRP, Inc. (Denver, PA).  
20 Western analysis of sera both from immunized rabbits and from immunized  
chickens demonstrated the presence of antibodies directed against the fusion  
protein.

To express GDF-8 in mammalian cells, the murine GDF-8 cDNA sequence from  
nucleotides 48-1303 was cloned in both orientations downstream of the  
25 metallothionein I promoter in the pMSXND expression vector; this vector  
contains processing signals derived from SV40, a dihydrofolate reductase  
gene, and a gene conferring resistance to the antibiotic G418 (Lee and

Nathans, J. Biol. Chem., 263:3521-3527). The resulting constructs were transfected into Chinese hamster ovary cells, and stable tranfectants were selected in the presence of G418. Two milliliters of conditioned media prepared from the G418-resistant cells were dialyzed, lyophilized, 5 electrophoresed under denaturing, reducing conditions, transferred to nitrocellulose, and incubated with anti-GDF-8 antibodies (described above) and [<sup>125</sup>I]iodoproteinA.

As shown in FIGURE 9, the rabbit GDF-8 antibodies (at a 1:500 dilution) detected a protein of approximately the predicted molecular weight for the 10 mature C-terminal fragment of GDF-8 in the conditioned media of cells transfected with a construct in which GDF-8 had been cloned in the correct (sense) orientation with respect to the metallothionein promoter (lane 2); this band was not detected in a similar sample prepared from cells transfected with a control antisense construct (lane 1). Similar results were obtained using 15 antibodies prepared in chickens. Hence, GDF-8 is secreted and proteolytically processed by these transfected mammalian cells.

#### EXAMPLE 5

#### EXPRESSION PATTERN OF GDF-8

To determine the pattern of GDF-8, 5  $\mu$ g of twice poly A-selected RNA 20 prepared from a variety of murine tissue sources were subjected to Northern analysis. As shown in FIGURE 10a (and as shown previously in Example 2), the GDF-8 probe detected a single mRNA species present almost exclusively in skeletal muscle among a large number of adult tissues surveyed. On longer exposures of the same blot, significantly lower but detectable levels of GDF-8 25 mRNA were seen in fat, brain, thymus, heart, and lung. Hence, these results confirm the high degree of specificity of GDF-8 expression in skeletal muscle. GDF-8 mRNA was also detected in mouse embryos at both gestational ages

(day 12.5 and day 18.5 post-coital) examined but not in placentas at various stages of development (FIGURE 10b).

**EXAMPLE 6**  
**CHROMOSOMAL LOCALIZATION OF GDF-8**

5 In order to map the chromosomal location of GDF-8, DNA samples from human/rodent somatic cell hybrids (Drwinga, et al., Genomics, 16:311-413, 1993; Dubois and Naylor, Genomics, 16:315-319, 1993) were analyzed by polymerase chain reaction followed by Southern blotting. Polymerase chain reaction was carried out using primer #83, 5'-  
10 CGCGGATCCGTGGATCTAAATGAGAACAGTGAGC-3' (SEQ ID NO:15) and primer #84, 5'-CGCGAATTCTCAGGTAATGATTGTTCCGTTGTAGCG-3'(SEQ ID NO:16) for 40 cycles at 94°C for 2 minutes, 60°C for 1 minute, and 72°C for 2 minutes. These primers correspond to nucleotides 119 to 143 (flanked by a Bam H1 recognition sequence), and nucleotides 394 to 418 (flanked by  
15 an Eco R1 recognition sequence), respectively, in the human GDF-8 cDNA sequence. PCR products were electrophoresed on agarose gels, blotted, and probed with oligonucleotide #100, 5'-ACACTAAATCTTCAAGAATA-3' (SEQ ID NO:17), which corresponds to a sequence internal to the region flanked by primer #83 and #84. Filters were hybridized in 6 X SSC, 1 X Denhardt's  
20 solution, 100 $\mu$ g/ml yeast transfer RNA, and 0.05% sodium pyrophosphate at 50°C.

As shown in FIGURE 11, the human-specific probe detected a band of the predicted size (approximately 320 base pairs) in the positive control sample (total human genomic DNA) and in a single DNA sample from the  
25 human/rodent hybrid panel. This positive signal corresponds to human chromosome 2. The human chromosome contained in each of the hybrid cell lines is identified at the top of each of the first 24 lanes (1-22, X, and Y). In the

lanes designated M, CHO, and H, the starting DNA template was total genomic DNA from mouse, hamster, and human sources, respectively. In the lane marked B1, no template DNA was used. Numbers at left indicate the mobilities of DNA standards. These data show that the human GDF-8 gene is located

5 on chromosome 2.

Although the invention has been described with reference to the presently preferred embodiment, it should be understood that various modifications can be made without departing from the spirit of the invention. Accordingly, the invention is limited only by the following claims.

**SUMMARY OF SEQUENCES**

SEQ ID NO: 1 is the nucleic acid sequence for clone SJL141.

SEQ ID NO: 2 is the nucleic acid sequence for clone SJL147.

SEQ ID NO: 3 is the nucleic acid sequence for clone ACM13.

5 SEQ ID NO: 4 is the nucleic acid sequence for clone ACM14.

SEQ ID NO: 5 is the partial nucleotide sequence and deduced amino acid sequence for murine GDF-8.

SEQ ID NO: 6 is the deduced partial amino acid sequence for murine GDF-8.

10 SEQ ID NO: 7 is the partial nucleotide sequence and deduced amino acid sequence for human GDF-8.

SEQ ID NO: 8 is the deduced partial amino acid sequence for human GDF-8.

SEQ ID NO: 9 is the amino acid sequence for primer SJL141.

SEQ ID NO: 10 is the amino acid sequence for primer SJL147.

15 SEQ ID NO: 11 is the nucleotide and deduced amino acid sequence for murine GDF-8.

SEQ ID NO: 12 is the deduced amino acid sequence for murine GDF-8.

SEQ ID NO: 13 is the nucleotide and deduced amino acid sequence for human GDF-8.

SEQ ID NO: 14 is the deduced amino acid sequence for human GDF-8.

5 SEQ ID NO's: 15 and 16 are nucleotide sequences for primer #83 and #84, respectively, which were used to map human GDF-8 in human/rodent somatic cell hybrids.

SEQ ID NO:17 is the nucleotide sequence of oligonucleotide #100 which corresponds to a sequence internal to the region flanked by primer #83 and #84.

## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

(i) APPLICANT: THE JOHNS HOPKINS UNIVERSITY

5 (ii) TITLE OF INVENTION: GROWTH DIFFERENTIATION FACTOR-8

(iii) NUMBER OF SEQUENCES: 17

(iv) CORRESPONDENCE ADDRESS:

10 (A) ADDRESSEE: Spensley Horn Jubas & Lubitz  
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(C) CITY: Los Angeles  
(D) STATE: California  
(E) COUNTRY: USA  
(F) ZIP: 90067

15 (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk  
(B) COMPUTER: IBM PC compatible  
(C) OPERATING SYSTEM: PC-DOS/MS-DOS  
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

20 (vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: PCT  
(B) FILING DATE: 18-MAR-1994  
(C) CLASSIFICATION:

(viii) ATTORNEY/AGENT INFORMATION:

25 (A) NAME: Wetherell, Jr., Ph.D., John R.,  
(B) REGISTRATION NUMBER: 31,678  
(C) REFERENCE/DOCKET NUMBER: FD-3413 CIP PCT

(ix) TELECOMMUNICATION INFORMATION:

30 (A) TELEPHONE: (619) 455-5100  
(B) TELEFAX: (619) 455-5110

## (2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 35 base pairs



-39-

- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

5 (vii) IMMEDIATE SOURCE:  
(B) CLONE: SJL141

(ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION: 1..35
- 10 (D) OTHER INFORMATION: /mod\_base= i  
/note= "B" is defined as "I" (inosine)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCGGAATTCC GBTGGVANRA YTGRTBRTB KCBCC  
35

15 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 33 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- 20 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:  
(B) CLONE: SJL147

(ix) FEATURE:

- (A) NAME/KEY: CDS
- 25 (B) LOCATION: 1..33

(ix) FEATURE:

- (A) NAME/KEY: modified\_base
- (B) LOCATION: 1..33
- 30 (D) OTHER INFORMATION: /mod\_base= i  
/note= "B" is defined as "I" (inosine)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CCGGAATTCC CABSCRCARC TNTCBACBRY CAT

33

(2) INFORMATION FOR SEQ ID NO:3:

- 5 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 32 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 10 (ii) MOLECULE TYPE: DNA (genomic)
- (vii) IMMEDIATE SOURCE:  
(B) CLONE: AC13
- (ix) FEATURE:  
(A) NAME/KEY: CDS  
15 (B) LOCATION: 1..32

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

CGCGGATCCA GAACTCAAGG TGACAGACAC AC

32

(2) INFORMATION FOR SEQ ID NO:4:

- 20 (i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 33 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear
- 25 (ii) MOLECULE TYPE: DNA (genomic)
- (vii) IMMEDIATE SOURCE:  
(B) CLONE: AC14
- (ix) FEATURE:

(A) NAME/KEY: CDS  
 (B) LOCATION: 1..33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGCGGATCCT CCTCATGAGC ACCCACAGCG GTC  
 5 33

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 550 base pairs  
 (B) TYPE: nucleic acid  
 10 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: mouse GDF-8

15 (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 59..436

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

TTAAGGTAGG AAGGATTTC A GGCTCTATTT ACATAATTGT TCTTTCCTTT TCACACAG  
 20 58

AAT CCC TTT TTA GAA GTC AAG GTG ACA GAC ACA CCC AAG AGG TCC CGG  
 106

Asn Pro Phe Leu Glu Val Lys Val Thr Asp Thr Pro Lys Arg Ser Arg  
 1 5 10 15

25 AGA GAC TTT GGG CTT GAC TGC GAT GAG CAC TCC ACG GAA TCC CGG TGC  
 154

Arg Asp Phe Gly Leu Asp Cys Asp Glu His Ser Thr Glu Ser Arg Cys  
 20 25 30

30 TGC CGC TAC CCC CTC ACG GTC GAT TTT GAA GCC TTT GGA TGG GAC TGG  
 202

Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala Phe Gly Trp Asp Trp  
 35 40 45

ATT ATC GCA CCC AAA AGA TAT AAG GCC AAT TAC TGC TCA GGA GAG TGT  
 250

5 Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr Cys Ser Gly Glu Cys  
 50 55 60

GAA TTT GTG TTT TTA CAA AAA TAT CCG CAT ACT CAT CTT GTG CAC CAA  
 298

10 Glu Phe Val Phe Leu Gln Lys Tyr Pro His Thr His Leu Val His Gln  
 65 70 75 80

GCA AAC CCC AGA GGC TCA GCA GGC CCT TGC TGC ACT CCG ACA AAA ATG  
 346

Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys Thr Pro Thr Lys Met  
 85 90 95

15 TCT CCC ATT AAT ATG CTA TAT TTT AAT GGC AAA GAA CAA ATA ATA TAT  
 394

Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly Lys Glu Gln Ile Ile Tyr  
 100 105 110

GGG AAA ATT CCA GCC ATG GTA GTA GAC CGC TGT GGG TGC TCA  
 436

20 Gly Lys Ile Pro Ala Met Val Val Asp Arg Cys Gly Cys Ser  
 115 120 125

TGAGCTTTGC ATTAGTTAG AAACTTCCCA AGTCATGGAA GGTCTTCCCC TCAATTTCGA  
 496

25 AACTGTGAAT TCCTGCAGCC CGGGGATCC ACTAGTTCTA GAGCGGCCGC CACC  
 550

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 126 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Asn Pro Phe Leu Glu Val Lys Val Thr Asp Thr Pro Lys Arg Ser Arg  
 1 5 10 15

Arg Asp Phe Gly Leu Asp Cys Asp Glu His Ser Thr Glu Ser Arg Cys  
 20 25 30

5 Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala Phe Gly Trp Asp Trp  
 35 40 45

Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr Cys Ser Gly Glu Cys  
 50 55 60

10 Glu Phe Val Phe Leu Gln Lys Tyr Pro His Thr His Leu Val His Gln  
 65 70 75 80

Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys Thr Pro Thr Lys Met  
 85 90 95

Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly Lys Glu Gln Ile Ile Tyr  
 100 105 110

15 Gly Lys Ile Pro Ala Met Val Val Asp Arg Cys Gly Cys Ser  
 115 120 125

(2) INFORMATION FOR SEQ ID NO:7:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 326 base pairs
  - (B) TYPE: nucleic acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: DNA (genomic)
- (vii) IMMEDIATE SOURCE:
  - (B) CLONE: human GDF-8
- (ix) FEATURE:
  - (A) NAME/KEY: CDS
  - (B) LOCATION: 3..326
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

-44-

CA AAA AGA TCC AGA AGG GAT TTT GGT CTT GAC TGT GAT GAG CAC TCA  
 47  
 Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His Ser  
 1 5 10 15

5 ACA GAA TCA CGA TGC TGT CGT TAC CCT CTA ACT GTG GAT TTT GAA GCT  
 95  
 Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu Ala  
 20 25 30

10 TTT GGA TGG GAT TGG ATT ATC GCT CCT AAA AGA TAT AAG GCC AAT TAC  
 143  
 Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn Tyr  
 35 40 45

TGC TCT GGA GAG TGT GAA TTT GTA TTT TTA CAA AAA TAT CCT CAT ACT  
 191  
 15 Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His Thr  
 50 55 60

CAT CTG GTA CAC CAA GCA AAC CCC AGA GGT TCA GCA GGC CCT TGC TGT  
 239  
 20 His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys Cys  
 65 70 75

ACT CCC ACA AAG ATG TCT CCA ATT AAT ATG CTA TAT TTT AAT GGC AAA  
 287  
 Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly Lys  
 80 85 90 95

25 GAA CAA ATA ATA TAT GGG AAA ATT CCA GCG ATG GTA GTA  
 326  
 Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val  
 100 105

## (2) INFORMATION FOR SEQ ID NO:8:

- 30 (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 108 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein



(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Gly Trp His Asp Trp Val Val Ala Pro  
1 5

(2) INFORMATION FOR SEQ ID NO:10:

5

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 8 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

10

(ii) MOLECULE TYPE: peptide

(vii) IMMEDIATE SOURCE:

(B) CLONE: SJL147

(ix) FEATURE:

15

(A) NAME/KEY: Peptide  
(B) LOCATION: 1..8  
(D) OTHER INFORMATION: /note= "Ile = Ile, Val, Met, Thr or  
Ala; Asp = Asp or Glu; Gly = Gly or Ala."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

20

Met Ile Val Asp Ser Cys Gly Cys  
1 5

(2) INFORMATION FOR SEQ ID NO:11:

25

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2676 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

(B) CLONE: Murine GDF-8



(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 104..1231

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

5 GTCTCTCGGA CGGTACATGC ACTAATATTT CACTTGGCAT TACTCAAAG CAAAAGAAG  
60

AAATAAGAAC AAGGAAAAA AAAAGATTGT GCTGATTTT AAA ATG ATG CAA AAA  
115

Met Met Gln Lys  
1

10 CTG CAA ATG TAT GTT TAT ATT TAC CTG TTC ATG CTG ATT GCT GCT GGC  
163

Leu Gln Met Tyr Val Tyr Ile Tyr Leu Phe Met Leu Ile Ala Ala Gly  
5 10 15 20

15 CCA GTG GAT CTA AAT GAG GGC AGT GAG AGA GAA GAA AAT GTG GAA AAA  
211

Pro Val Asp Leu Asn Glu Gly Ser Glu Arg Glu Glu Asn Val Glu Lys  
25 30 35

20 GAG GGG CTG TGT AAT GCA TGT GCG TGG AGA CAA AAC ACG AGG TAC TCC  
259

Glu Gly Leu Cys Asn Ala Cys Ala Trp Arg Gln Asn Thr Arg Tyr Ser  
40 45 50

AGA ATA GAA GCC ATA AAA ATT CAA ATC CTC AGT AAG CTG CGC CTG GAA  
307

25 Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu Arg Leu Glu  
55 60 65

ACA GCT CCT AAC ATC AGC AAA GAT GCT ATA AGA CAA CTT CTG CCA AGA  
355

30 Thr Ala Pro Asn Ile Ser Lys Asp Ala Ile Arg Gln Leu Leu Pro Arg  
70 75 80

CGG CCT CCA CTC CGG GAA CTG ATC GAT CAG TAC GAC GTC CAG AGG GAT  
403

Ala Pro Pro Leu Arg Glu Leu Ile Asp Gln Tyr Asp Val Gln Arg Asp  
85 90 95 100

-48-

GAC AGC AGT GAT GGC TCT TTG GAA GAT GAC GAT TAT CAC GCT ACC ACG  
 451  
 Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr His Ala Thr Thr  
                   105                                  110                                  115

5 GAA ACA ATC ATT ACC ATG CCT ACA GAG TCT GAC TTT CTA ATG CAA GCG  
 499  
 Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe Leu Met Gln Ala  
                   120                                  125                                  130

10 GAT GGC AAG CCC AAA TGT TGC TTT TTT AAA TTT AGC TCT AAA ATA CAG  
 547  
 Asp Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser Lys Ile Gln  
                   135                                  140                                  145

15 TAC AAC AAA GTA GTA AAA GCC CAA CTG TGG ATA TAT CTC AGA CCC GTC  
 595  
 Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr Leu Arg Pro Val  
                   150                                  155                                  160

20 AAG ACT CCT ACA ACA GTG TTT GTG CAA ATC CTG AGA CTC ATC AAA CCC  
 643  
 Lys Thr Pro Thr Thr Val Phe Val Gln Ile Leu Arg Leu Ile Lys Pro  
                   165                                  170                                  175                                  180

25 ATG AAA GAC GGT ACA AGG TAT ACT GGA ATC CGA TCT CTG AAA CTT GAC  
 691  
 Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu Lys Leu Asp  
                                   185                                  190                                  195

30 ATG AGC CCA GGC ACT GGT ATT TGG CAG AGT ATT GAT GTG AAG ACA GTG  
 739  
 Met Ser Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val Lys Thr Val  
                   200                                  205                                  210

35 TTG CAA AAT TGG CTC AAA CAG CCT GAA TCC AAC TTA GGC ATT GAA ATC  
 787  
 Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly Ile Glu Ile  
                   215                                  220                                  225

AAA GCT TTG GAT GAG AAT GGC CAT GAT CTT GCT GTA ACC TTC CCA GGA  
 835  
 Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr Phe Pro Gly  
                   230                                  235                                  240

CCA GGA GAA GAT GGG CTG AAT CCC TTT TTA GAA GTC AAG GTG ACA GAC  
 883  
 Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys Val Thr Asp  
 245 250 255 260

5 ACA CCC AAG AGG TCC CGG AGA GAC TTT GGG CTT GAC TGC GAT GAG CAC  
 931  
 Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys Asp Glu His  
 265 270 275

10 TCC ACG GAA TCC CGG TGC TGC CGC TAC CCC CTC ACG GTC GAT TTT GAA  
 979  
 Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val Asp Phe Glu  
 280 285 290

15 GCC TTT GGA TGG GAC TGG ATT ATC GCA CCC AAA AGA TAT AAG GCC AAT  
 1027  
 Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr Lys Ala Asn  
 295 300 305

20 TAC TGC TCA GGA GAG TGT GAA TTT GTG TTT TTA CAA AAA TAT CCG CAT  
 1075  
 Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys Tyr Pro His  
 310 315 320

ACT CAT CTT GTG CAC CAA GCA AAC CCC AGA GGC TCA GCA GGC CCT TGC  
 1123  
 Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala Gly Pro Cys  
 325 330 335 340

25 TGC ACT CCG ACA AAA ATG TCT CCC ATT AAT ATG CTA TAT TTT AAT GGC  
 1171  
 Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr Phe Asn Gly  
 345 350 355

30 AAA GAA CAA ATA ATA TAT GGG AAA ATT CCA GCC ATG GTA GTA GAC GGC  
 1219  
 Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val Val Asp Arg  
 360 365 370

35 TGT GGG TGC TCA TGAGCTTTGC ATTAGGTTAG AAACTTCCCA AGTCATGGAA  
 1271  
 Cys Gly Cys Ser  
 375

GGTCTTCCCC TCAATTTCGA AACTGTGAAT TCAAGCACCA CAGGCTGTAG GCCTTGAGTA  
 1331

TGCTCTAGTA ACGTAAGCAC AAGCTACAGT GTATGAACTA AAAGAGAGAA TAGATGCAAT  
 1391

5 GGTTGGCATT CAACCACCAA AATAAACCAT ACTATAGGAT GTTGTATGAT TTCCAGAGTT  
 1451

TTTGAAATAG ATGGAGATCA AATTACATTT ATGTCCATAT ATGTATATTA CAACTACAAT  
 1511

10 CTAGGCAAGG AAGTGAGAGC ACATCTTGTG GTCTGCTGAG TTAGGAGGGT ATGATTAAAA  
 1571

GGTAAAGTCT TATTTCTTAA CAGTTTCACT TAATATTTAC AGAAGAATCT ATATGTAGCC  
 1631

TTTGTAAAGT GTAGGATTGT TATCATTTAA AAACATCATG TACACTTATA TTTGTATTGT  
 1691

15 ATACTTGGTA AGATAAAATT CCACAAAGTA GGAATGGGGC CTCACATACA CATTGCCATT  
 1751

CCTATTATAA TTGGACAATC CACCACGGTG CTAATGCAGT GCTGAATGGC TCCTACTGGA  
 1811

20 CCTCTCGATA GAACACTCTA CAAAGTACGA GTCTCTCTCT CCCTTCCAGG TGCATCTCCA  
 1871

CACACACAGC ACTAAGTGTT CAATGCATT TCTTTAAGGA AAGAAGAATC TTTTTTCTA  
 1931

GAGGTCAACT TTCAGTCAAC TCTAGCACAG CGGGAGTGAC TGCTGCATCT TAAAAGGCAG  
 1991

25 CCAAACAGTA TTCATTTTTT AATCTAAATT TCAAAATCAC TGCTGCCTT TATCACATGG  
 2051

CAATTTTGTG GTAAAATAAT GCAAATGACT GGTCTATCA ATATTGTATA AAAGACTCTG  
 2111

30 AAACAATTAC ATTIATATAA TATGTATACA ATATTGTTTT GTAAATAAGT GTCTCCTTTT  
 2171

ATATTTACTT TGGTATATTT TTACACTAAT GAAATTTCAA ATCATTAAAG TACAAAGACA  
2231

TGTCATGTAT CACAAAAAAG GTGACTGCTT CTATTTTCAGA GTGAATTAGC AGATTCAATA  
2291

5 GTGGTCTTAA AACTCTGTAT GTTAAGATTA GAAGGTATA TTACAATCAA TTTATGTATT  
2351

TTTTACATTA TCAACTTATG GTTTCATGGT GGCTGTATCT ATGAATGTGG CTCCCAGTCA  
2411

10 AATTTCAATG CCCCAACCATT TTAAAAATTA CAAGCATTAC TAAACATACC AACATGTATC  
2471

TAAAGAAATA CAAATATGGT ATCTCAATAA CAGCTACTTT TTTATTTTAT AATTGACAA  
2531

TGAATACATT TCTTTTATTT ACTTCAGTTT TATAAATTGG AACTTTGTTT ATCAAATGTA  
2591

15 TTGTACTCAT AGCTAAATGA AATTATTTCT TACATAAAAA TGTGTAGAAA CTATAAATTA  
2651

AAGTGTTTTC ACATTTTGA AAGGC  
2676

(2) INFORMATION FOR SEQ ID NO:12:

20 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 376 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Met Met Gln Lys Leu Gln Met Tyr Val Tyr Ile Tyr Leu Phe Met Leu  
 1 5 10 15

Ile Ala Ala Gly Pro Val Asp Leu Asn Glu Gly Ser Glu Arg Glu Glu  
 20 25 30



Cys Asp Glu His Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr  
 275 280 285

Val Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg  
 290 295 300

5 Tyr Lys Ala Asn Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln  
 305 310 315 320

Lys Tyr Pro His Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser  
 325 330 335

10 Ala Gly Pro Cys Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu  
 340 345 350

Tyr Phe Asn Gly Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met  
 355 360 365

Val Val Asp Arg Cys Gly Cys Ser  
 370 375

15 (2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2743 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:  
 (B) CLONE: Human GDF-8

25

(ix) FEATURE:  
 (A) NAME/KEY: CDS  
 (B) LOCATION: 59..1183

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AAGAAAAGTA AAAGGAAGAA ACAAGAACAA GAAAAAAGAT TATATTGATT TAAAAATC  
 58

ATG CAA AAA CTG CAA CTC TGT GTT TAT ATT TAC CTG TTT ATG CTG ATT  
 106  
 Met Gln Lys Leu Gln Leu Cys Val Tyr Ile Tyr Leu Phe Met Leu Ile  
 1 5 10 15  
  
 5 GTT GCT GGT CCA GTG GAT CTA AAT GAG AAC AGT GAG CAA AAA GAA AAT  
 154  
 Val Ala Gly Pro Val Asp Leu Asn Glu Asn Ser Glu Gln Lys Glu Asn  
 20 25 30  
  
 10 GTG GAA AAA GAG GGG CTG TGT AAT GCA TGT ACT TGG AGA CAA AAC ACT  
 202  
 Val Glu Lys Glu Gly Leu Cys Asn Ala Cys Thr Trp Arg Gln Asn Thr  
 35 40 45  
  
 AAA TCT TCA AGA ATA GAA GCC ATT AAG ATA CAA ATC CTC AGT AAA CTT  
 250  
 15 Lys Ser Ser Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu  
 50 55 60  
  
 CGT CTG GAA ACA GCT CCT AAC ATC AGC AAA GAT GTT ATA AGA CAA CTT  
 298  
 Arg Leu Glu Thr Ala Pro Asn Ile Ser Lys Asp Val Ile Arg Gln Leu  
 20 65 70 75 80  
  
 TTA CCC AAA GCT CCT CCA CTC CGG GAA CTG ATT GAT CAG TAT GAT GTC  
 346  
 Leu Pro Lys Ala Pro Pro Leu Arg Glu Leu Ile Asp Gln Tyr Asp Val  
 85 90 95  
  
 25 CAG AGG GAT GAC AGC AGC GAT GGC TCT TTG GAA GAT GAC GAT TAT CAC  
 394  
 Gln Arg Asp Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr His  
 100 105 110  
  
 GCT ACA ACG GAA ACA ATC ATT ACC ATG CCT ACA GAG TCT GAT TTT CTA  
 442  
 30 Ala Thr Thr Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe Leu  
 115 120 125  
  
 ATG CAA GTG GAT GGA AAA CCC AAA TGT TGC TTC TTT AAA TTT AGC TCT  
 490  
 35 Met Gln Val Asp Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser  
 130 135 140



-55-

AAA ATA CAA TAC AAT AAA GTA GTA AAG GCC CAA CTA TGG ATA TAT TTG  
 538  
 Lys Ile Gln Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr Leu  
 145 150 155 160

5 AGA CCC GTC GAG ACT CCT ACA ACA GTG TTT GTG CAA ATC CTG AGA CTC  
 586  
 Arg Pro Val Glu Thr Pro Thr Thr Val Phe Val Gln Ile Leu Arg Leu  
 165 170 175

10 ATC AAA CCT ATG AAA GAC GGT ACA AGG TAT ACT GGA ATC CGA TCT CTG  
 634  
 Ile Lys Pro Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu  
 180 185 190

15 AAA CTT GAC ATG AAC CCA GGC ACT GGT ATT TGG CAG AGC ATT GAT GTG  
 682  
 Lys Leu Asp Met Asn Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val  
 195 200 205

20 AAG ACA GTG TTG CAA AAT TGG CTC AAA CAA CCT GAA TCC AAC TTA GGC  
 730  
 Lys Thr Val Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly  
 210 215 220

ATT GAA ATA AAA GCT TTA GAT GAG AAT GGT CAT GAT CTT GCT GTA ACC  
 778  
 Ile Glu Ile Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr  
 225 230 235 240

25 TTC CCA GGA CCA GGA GAA GAT GGG CTG AAT CCG TTT TTA GAG GTC AAG  
 826  
 Phe Pro Gly Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys  
 245 250 255

30 GTA ACA GAC ACA CCA AAA AGA TCC AGA AGG GAT TTT GGT CTT GAC TGT  
 874  
 Val Thr Asp Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys  
 260 265 270

35 GAT GAG CAC TCA ACA GAA TCA CGA TGC TGT CGT TAC CCT CTA ACT GTG  
 922  
 Asp Glu His Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val  
 275 280 285

GAT TTT GAA GCT TTT GGA TGG GAT TGG ATT ATC GCT CCT AAA AGA TAT  
 970  
 Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr  
           290                                  295                                  300

5 AAG GCC AAT TAC TGC TCT GGA GAG TGT GAA TTT GTA TTT TTA CAA AAA  
 1018  
 Lys Ala Asn Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys  
           305                                  310                                  315                                  320

10 TAT CCT CAT ACT CAT CTG GTA CAC CAA GCA AAC CCC AGA GGT TCA GCA  
 1066  
 Tyr Pro His Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala  
                                   325                                  330                                  335

15 GGC CCT TGC TGT ACT CCC ACA AAG ATG TCT CCA ATT AAT ATG CTA TAT  
 1114  
 Gly Pro Cys Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr  
                                   340                                  345                                  350

20 TTT AAT GGC AAA GAA CAA ATA ATA TAT GGG AAA ATT CCA GCG ATG GTA  
 1162  
 Phe Asn Gly Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val  
                                   355                                  360                                  365

GTA GAC CGC TGT GGG TGC TCA TGAGATTAT ATTAAGCGTT CATAACTTCC  
 1213  
 Val Asp Arg Cys Gly Cys Ser  
           370                                  375

25 TAAACATGG AAGTTTTTC CCTCAACAAT TTGAAGCTG TGAAATTAAG TACCACAGGC  
 1273

TATAGGCCTA GAGTATGCTA CAGTCACTTA AGCATAAGCT ACAGTATGTA AACTAAAAGG  
 1333

30 GGGAATATAT GCAATGGTTG GCATTTAACC ATCCAAACAA ATCATACAAG AAAGTTTTAT  
 1393

GATTTCCAGA GTTTTGAGC TAGAAGGAGA TCAAATTACA TTTATGTTCC TATATATTAC  
 1453

AACATCGCG AGGAAATGAA AGCGATTCTC CTGAGTTCT GATGAATTAA AGGAGTATGC  
 1513

TTAAAGTCT ATTTCTTAA AGTTTTGTT AATATTTACA GAAAAATCCA CATACAGTAT  
 1573

TGGTAAAATG CAGGATTGTT ATATACCATC ATTCGAATCA TCCTTAAACA CTGAATTTA  
 1633

5 TATTGTATGG TAGTATACTT GGTAAGATAA AATTCACAA AAATAGGGAT GGTGCAGCAT  
 1693

ATGCAATTC CATTCTTATT ATAATTGACA CAGTACATTA ACAATCCATG CCAACGGTGC  
 1753

10 TAATACGATA GGCTGAATGT CTGAGGCTAC CAGGTTTATC ACATAAAAAA CATTGAGTAA  
 1813

AATAGTAACT TTCTCTTTTC TTCAGGTGCA TTTTCCTACA CCTCCAAATG AGGAATGGAT  
 1873

TTTCTTTAAT GTAAGAAGAA TCATTTTCT AGAGGTTGGC TTTCAATTCT GTAGCATACT  
 1933

15 TGGAGAAACT GCATTATCTT AAAAGGCAGT CAAATGGTGT TTGTTTTTAT CAAAATGTCA  
 1993

AAATAACATA CTGGAGAAG TATGTAATTT TGTCTTTGGA AAATTACAAC ACTGCCTTTG  
 2053

20 CAACACTGCA GTTTTTATGG TAAAATAATA GAAATGATCG ACTCTATCAA TATTGTATAA  
 2113

AAAGACTGAA ACAATGCATT TATATAATAT GTATACAATA TTGTTTTGTA AATAAGTGTC  
 2173

TCCTTTTTTA TTTACTTTGG TATATTTTA CACTAAGGAC ATTCAAATT AAGTACTAAG  
 2233

25 GCACAAAGAC ATGTCATGCA TCACAGAAAA GCAACTACTT ATATTCAGA GCAAATTAGC  
 2293

AGATTAAATA GTGGTCTTAA AACTCCATAT GTTAATGATT AGATGGTTAT ATTACAATCA  
 2353

30 TTTTATATTT TTTTACATGA TTAACATTCA CTTATGGATT CATGATGGCT GTATAAAGTC  
 2413

AATTTGAAAT TTCAATGGTT TACTGTCATT GTGTTTAAAT CTCAACGTC CATTATTTTA  
2473

ATACTTGCAA AACATTACT AAGTATACCA AAATAATTGA CTCTATTATC TGAATGAAG  
2533

5 AATAAACTGA TGCTATCTCA ACAATAACTG TTACTTTTAT TTTATAATTT GATAATGAAT  
2593

ATATTTCTGC ATTTATTAC TTCTGTTTTG TAAATTGGGA TTTTGTTAAT CAAATTTATT  
2653

10 GTACTATGAC TAAATGAAAT TATTTCTTAC ATCTAATTTG TAGAAACAGT ATAAGTTATA  
2713

TTAAAGTGTT TTCACATTTT TTTGAAAGAC  
2743

(2) INFORMATION FOR SEQ ID NO:14:

- 15 (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 375 amino acids
  - (B) TYPE: amino acid
  - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

20 Met Gln Lys Leu Gln Leu Cys Val Tyr Ile Tyr Leu Phe Met Leu Ile  
1 5 10 15

Val Ala Gly Pro Val Asp Leu Asn Glu Asn Ser Glu Gln Lys Glu Asn  
20 25 30

25 Val Glu Lys Glu Gly Leu Cys Asn Ala Cys Thr Trp Arg Gln Asn Thr  
35 40 45

Lys Ser Ser Arg Ile Glu Ala Ile Lys Ile Gln Ile Leu Ser Lys Leu  
50 55 60

Arg Leu Glu Thr Ala Pro Asn Ile Ser Lys Asp Val Ile Arg Gln Leu  
65 70 75 80

Leu Pro Lys Ala Pro Pro Leu Arg Glu Leu Ile Asp Gln Tyr Asp Val  
 85 90 95

Gln Arg Asp Asp Ser Ser Asp Gly Ser Leu Glu Asp Asp Asp Tyr His  
 100 105 110

5 Ala Thr Thr Glu Thr Ile Ile Thr Met Pro Thr Glu Ser Asp Phe Leu  
 115 120 125

Met Gln Val Asp Gly Lys Pro Lys Cys Cys Phe Phe Lys Phe Ser Ser  
 130 135 140

10 Lys Ile Gln Tyr Asn Lys Val Val Lys Ala Gln Leu Trp Ile Tyr Leu  
 145 150 155 160

Arg Pro Val Glu Thr Pro Thr Thr Val Phe Val Gln Ile Leu Arg Leu  
 165 170 175

Ile Lys Pro Met Lys Asp Gly Thr Arg Tyr Thr Gly Ile Arg Ser Leu  
 180 185 190

15 Lys Leu Asp Met Asn Pro Gly Thr Gly Ile Trp Gln Ser Ile Asp Val  
 195 200 205

Lys Thr Val Leu Gln Asn Trp Leu Lys Gln Pro Glu Ser Asn Leu Gly  
 210 215 220

20 Ile Glu Ile Lys Ala Leu Asp Glu Asn Gly His Asp Leu Ala Val Thr  
 225 230 235 240

Phe Pro Gly Pro Gly Glu Asp Gly Leu Asn Pro Phe Leu Glu Val Lys  
 245 250 255

Val Thr Asp Thr Pro Lys Arg Ser Arg Arg Asp Phe Gly Leu Asp Cys  
 260 265 270

25 Asp Glu His Ser Thr Glu Ser Arg Cys Cys Arg Tyr Pro Leu Thr Val  
 275 280 285

Asp Phe Glu Ala Phe Gly Trp Asp Trp Ile Ile Ala Pro Lys Arg Tyr  
 290 295 300

30 Lys Ala Asn Tyr Cys Ser Gly Glu Cys Glu Phe Val Phe Leu Gln Lys  
 305 310 315 320

-60-

Tyr Pro His Thr His Leu Val His Gln Ala Asn Pro Arg Gly Ser Ala  
 325 330 335

Gly Pro Cys Cys Thr Pro Thr Lys Met Ser Pro Ile Asn Met Leu Tyr  
 340 345 350

5 Phe Asn Gly Lys Glu Gln Ile Ile Tyr Gly Lys Ile Pro Ala Met Val  
 355 360 365

Val Asp Arg Cys Gly Cys Ser  
 370 375

## (2) INFORMATION FOR SEQ ID NO:15:

- 10 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 34 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear
- 15 (ii) MOLECULE TYPE: DNA (genomic)
- (vii) IMMEDIATE SOURCE:  
 (B) CLONE: #83
- (ix) FEATURE:  
 (A) NAME/KEY: CDS  
 20 (B) LOCATION: 1..34

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGCGGATCCG TGGATCTAAA TGAGAACAGT GAGC  
 34

## (2) INFORMATION FOR SEQ ID NO:16:

- 25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 37 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

-61-

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

(B) CLONE: #84

(ix) FEATURE:

5

(A) NAME/KEY: CDS

(B) LOCATION: 1..37

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGCGAATTCT CAGGTAATGA TTGTTCCGT TGTAGCC  
37

10 (2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 20 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: single

15

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(vii) IMMEDIATE SOURCE:

(B) CLONE: #100

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

20 AACTAAATC TTCAAGAATA  
20

**CLAIMS**

1. Substantially pure growth differentiation factor-8 (GDF-8) and functional fragments thereof.
2. An isolated polynucleotide sequence encoding the GDF-8 polypeptide of claim 1.
3. The polynucleotide of claim 2, wherein the GDF-8 nucleotide sequence is selected from the group consisting of the nucleic acid sequence of
  - a. FIGURE 5a, wherein T can also be U;
  - b. FIGURE 5b, wherein T can also be U;
  - 5 c. nucleic acid sequences complementary to FIGURE 5a;
  - d. nucleic acid sequences complementary to FIGURE 5b;
  - e. fragments of a. or c. that are at least 15 bases in length and that will selectively hybridize to genomic DNA which encodes the GDF-8 protein of FIGURE 5a; and
  - 10 f. fragments of b. or d. that are at least 15 bases in length and that will selectively hybridize to genomic DNA which encodes the GDF-8 protein of FIGURE 5b.
4. The polynucleotide sequence of claim 2, wherein the polynucleotide is isolated from a mammalian cell.
5. The polynucleotide of claim 4, wherein the mammalian cell is selected from the group consisting of mouse, rat, and human cell.
6. An expression vector including the polynucleotide of claim 2.
7. The vector of claim 6, wherein the vector is a plasmid.



8. The vector of claim 6, wherein the vector is a virus.
9. A host cell stably transformed with the vector of claim 6.
10. The host cell of claim 9, wherein the cell is prokaryotic.
11. The host cell of claim 9, wherein the cell is eukaryotic.
12. Antibodies reactive with the polypeptide of claim 1 or fragments thereof.
13. The antibodies of claim 12, wherein the antibodies are polyclonal.
14. The antibodies of claim 12, wherein the antibodies are monoclonal.
15. A method of detecting a cell proliferative disorder comprising contacting the antibody of claim 12 with a specimen of a subject suspected of having a GDF-8 associated disorder and detecting binding of the antibody.
16. The method of claim 15, wherein the cell is a muscle cell.
17. The method of claim 15, wherein the detecting is in vivo.
18. The method of claim 17, wherein the antibody is detectably labeled.
19. The method of claim 18, wherein the detectable label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound and a chemiluminescent compound.
20. The method of claim 15, wherein the detection is in vitro.

21. The method of claim 20, wherein the antibody is detectably labeled.
22. The method of claim 21, wherein the label is selected from the group consisting of a radioisotope, a fluorescent compound, a bioluminescent compound, a chemoluminescent compound and an enzyme.
23. A method of treating a cell proliferative disorder associated with expression of GDF-8, comprising contacting the cells with a reagent which suppresses the GDF-8 activity.
24. The method of claim 23, wherein the reagent is an anti-GDF-8 antibody.
25. The method of claim 23, wherein the reagent is a GDF-8 antisense sequence.
26. The method of claim 23, wherein the cell is a muscle cell.
27. The method of claim 23, wherein the reagent which suppresses GDF-8 activity is introduced to a cell using a vector.
28. The method of claim 27, wherein the vector is a colloidal dispersion system.
29. The method of claim 28, wherein the colloidal dispersion system is a liposome.
30. The method of claim 29, wherein the liposome is essentially target specific.
31. The method of claim 30, wherein the liposome is anatomically targeted.

32. The method of claim 31, wherein the liposome is mechanistically targeted.
33. The method of claim 32, wherein the mechanistic targeting is passive.
34. The method of claim 32, wherein the mechanistic targeting is active.
35. The method of claim 34, wherein the liposome is actively targeted by coupling with a moiety selected from the group consisting of a sugar, a glycolipid, and a protein.
36. The method of claim 35, wherein the protein moiety is an antibody.
37. The method of claim 36, wherein the vector is a virus.
38. The method of claim 37, wherein the virus is an RNA virus.
39. The method of claim 38, wherein the RNA virus is a retrovirus.
40. The method of claim 39, wherein the retrovirus is essentially target specific.
41. The method of claim 40, wherein a moiety for target specificity is encoded by a polynucleotide inserted into the retroviral genome.
42. The method of claim 40, wherein a moiety for target specificity is selected from the group consisting of a sugar, a glycolipid, and a protein.
43. The method of claim 42, wherein the protein is an antibody.

HEART  
LUNG  
THYMUS  
BRAIN  
KIDNEY  
SEMINAL VESICLE  
PANCREAS  
INTESTINE  
SPLEEN  
TESTIS  
FAT  
UTERUS  
OVARY  
LIVER  
MUSCLE

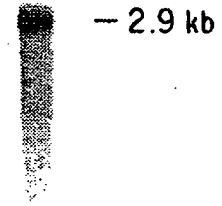


FIG. 1

1 TTAAGGTAGGAAGGATTT CAGGCTCTATTTACATAATTGTTCTTTTCCTTTTCACACAGAA 60  
 N  
 61 TCCCTTTTITAGAAGTCAAGGTGACAGACACACCCCAAGAGGTCCCGGAGAGACTTTGGGCT 120  
 P F L E V K V T D T P **K R** S **R R** D F G L  
 121 TCACTGCGATGAGCACTCCACGGAATCCCGGTGCTCCCGCTACCCCTCAGGTCGATTT 180  
 D C D E H S T E S R C C R Y P L T V D F  
 181 TGAAGCCTTTGGATGGGACTGGATTATCCACCCAAAAGATATAAGGCCAATTACTGCTC 240  
 E A F G W D W I I A P K R Y K A N Y C S  
 241 AGGAGAGTGTGAATTTGTGTTTTACAAAAATATCCGCATACTCATCTTGTGCACCAAGC 300  
 G E C E F V F L Q K Y P H T H L V H Q A  
 301 AAACCCAGAGGCTCAGCAGGCCCTTGCTGCACTCCGACAAAAATGTCTCCCATTAATAT 360  
 N P R G S A G P C C T P T K M S P I N M  
 361 GCTATATTTAATGGCAAAGAACAATAATATATGGGAAAATCCAGCCATGGTAGTAGA 420  
 L Y F N G K E Q I I Y G K I P A M V V D  
 421 CCGCTGTGGGTGCTCATGAGCTTTGCATTAGGTTAGAAACTCCCAAGTCATGGAAGGTC 480  
 R C G C S \*  
 481 TTCCCTCAATTTGAAACTGTGAATTCCTGCAGCCCGGGGATCCACTAGTTCTAGAGC 540  
 541 GGCCGCCACC 550

FIG.2a

1 CAAAAAGATCCAGAAGGGATTTTGGTCTTACTGTGATGAGCACTCAACAGAATCAGGAT 60  
**K R** S **R R** D F G L D C D E H S T E S R C  
 61 GCTGTGTTACCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATGGATTATCGCTC 120  
 C R Y P L T V D F E A F G W D W I I A P  
 121 CTAAGATATAAGGCCAATTACTGCTCTGGAGAGTGTGAATTTGTATTTTACAAAAAT 180  
 K R Y K A N Y C S G E C E F V F L Q K Y  
 181 ATCCTCATACTCATCTGGTACACCAAGCAAACCCAGAGGTTACAGAGGCCCTTGCTGTA 240  
 P H T H L V H Q A N P R G S A G P C C T  
 241 CTCCACAAAGATGTCTCCAATTAATATGCTATATTTAATGGCAAAGAACAATAATAT 300  
 P T K M S P I N M L Y F N G K E Q I I Y  
 301 ATGGGAAAATCCAGCGATGGTAGTA 326  
 G K I P A M V V

FIG.2b

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GDF-8 SRRDFGLDCDEHSTESRCCRYPLTVDF-EAFGWD-WI IAPKRYKANYCSGECFVFLQKYP—  
 GDF-1 RPRRDAEVLGGPGGACRARRLYVSF-REVGWHRWV IAPRGFLANYCOGCOALPVALSGSGCPP  
 BMP-2 REKRQAKHKQRKRLKSSCKRHPLYVDF-SDVGWNDWI VAPPGYHAFYCHGECPPFLADHLNS—  
 BMP-4 KRSPKHHSQRARKKNKNCRRHSLYVDF-SDVGWNDWI VAPPGYQAFYCHGDCPPFLADHLNS—  
 Vgr-1 SRGSGSSDYNGSELKTACKKHELVSF-QDLGWQDWI IAPKGYAANYCDGECSPFLNAHMNA—  
 OP-1 LRMANVAENSSSDQROACKKHELVSF-RDLGWQDWI IAPEGYAAYYCEGECAPFLNSYMNA—  
 BMP-5 SRMSSVGDYNTSEKQKACKKHELVSF-RDLGWQDWI IAPEGYAAFYCDGECSPFLNAHMNA—  
 BMP-3 EQTLKKARRKQWIEPRNCAARYLKVDF-ADIGWSEWI IAPKSFDAAYYCSGACQFPMPSLKPS—  
 MIS GPGRAQRSAGATAADGPCALRELSVDL—RAERSVLIPE TYQANNCOGVCGWQSDRNPY—  
 Inhibin $\alpha$  ALRLLQRPPEEPAAHANCHRVALNLSF-QELGWERWIVYPPSFIFHYCHGGGLHIPPNSLSPV-  
 Inhibin $\beta$ A HRRRRRGLCDGKV-NICCKKQFFVSF-KDIGWNDWI IAPSGYHANYCEGECPSH IAGTSGSSL-  
 Inhibin $\beta$ B HRIRKRGLECDGRT-NLCCRQOFFIDF-RLIGWNDWI IAPTGYGNYCEGSCPAYLAGVPGSAS-  
 TGF- $\beta$ 1 HRRALDTNYCF SSTEKNCCVRQLYIDFRKDLGWK-WIHEPKGYHANFOLGPOPIWSLD—  
 TGF- $\beta$ 2 KKRALDAAYCF RNVQDNCLRPLYIDFRKDLGWK-WIHEPKGYANFAGACPYLWSSD—  
 TGF- $\beta$ 3 KKRALDTNYCF RNLEENCCVRPLYIDFRQDLGWK-WVHEPKGYANFCSGPOPYLRSAD—

GDF-8 -HTHLVHQANPRG—SAGPCCT—PTKMSPINMLYF-NGKEQI IYGKI PAMVVDRCGCS  
 GDF-1 ALNHAVALRALMHA—AAPGAADLPCCV—PARLSPI SVLFF-DNSDNVVLROYEDMVVDECCGR  
 BMP-2 -TNHAI VQTLVNS—VNSKIPKACCV—PTELSAISMLYL-DENEKVVLKNYQDMVVEGCGCR  
 BMP-4 -TNHAI VQTLVNS—VNSSIPKACCV—PTELSAISMLYL-DEYDKVVLKNYQDMVVEGCGCR  
 Vgr-1 -TNHAI VQTLVHL—MNPEYVPKPCA—PTKLNAISVLYF-DDNSNVLKKYRNMVVRACGCH  
 OP-1 -TNHAI VQTLVHF—INPETVPKPCA—PTQLNAISVLYF-DDSSNVLKKYRNMVVRACGCH  
 BMP-5 -TNHAI VQTLVHL—MFDHVPKPCA—PTKLNAISVLYF-DDSSNVLKKYRNMVVRSGGCH  
 BMP-3 -NHATIQSIVRA-VGVVPGIPEPCCV—PEKMSSLSILFF-DENKNVVLKVYPNMTVE SCACR  
 MIS -GNHVLL LKMQA—RGAALARPPCCV—PTAYAGKLL I SLSEER—ISAHVPMNVATECCGR  
 Inhibin $\alpha$  -PGAPPTPAQPYS—LLPGAQPCAALPGTMRPLHVRTTSDGGYSFKYE TVPNLLTQHACI  
 Inhibin $\beta$ A -SFHSTVINHYMRGHSPFANLKSCCV—PTKLRPMSMLYF-DDGQNI I KKDIONMIVEEGGCS  
 Inhibin $\beta$ B -SFHTAVNQYRMRLNPGT-VNSCCI—PTKLSTMSMLYF-DDEYNIVKRDVPMI VE EGGCA  
 TGF- $\beta$ 1 -TQYSKVLALYNO—HNPGASAAPCCV—POALEPLPIVYY-VGRKPKV-EQLSNMIVRSCKCS  
 TGF- $\beta$ 2 -TQHSRVL SLYNT—INPEASASPCCV—SQDLEPLTILYY-IGKTPKI-EQLSNMIVKSKCS  
 TGF- $\beta$ 3 -TTHSTVLGLYNT—LNPEASASPCCV—PQDLEPLTILYY-VGRTPKV-EQLSNMIVKSKCS

FIG.3







1261 AAGTCATGGAAGGTCTTCCCCTCAATTTGAAACTGTGAATTCAAGCACCACAGGCTGTA 1320  
1321 GGCCTTGAGTATGCTCTAGTAACGTAAGCACAAGCTACAGTGTATGAACTAAAAGAGAGA 1380  
1381 ATAGATGCAATGGTTGGCATTCAACCACCAAATAAACCATACTATAGGATGTTGTATGA 1440  
1441 TTTCCAGAGTTTTTGAATAGATGGAGATCAAATTACATTTATGTCCATATATGTATATT 1500  
1501 ACAACTACAATCTAGGCAAGGAAGTGAGAGCACATCTTGTGGTCTGCTGAGTTAGGAGGG 1560  
1561 TATGATTAAGGTAAGTCTTATTTCTAACAGTTTCACTTAATATTTACAGAAGAATC 1620  
1621 TATATGTAGCCTTTGTAAGTGTAGGATTGTATCATTAAAAACATCATGTACACTTAT 1680  
1681 ATTTGTATTGTATACTTGGTAAGATAAAATCCACAAAGTAGGAATGGGGCCTCACATAC 1740  
1741 ACATTGCCATTCCATTATAATGGACAATCCACCACGGTCTAATGCAGTGCTGAATGG 1800  
1801 CTCCTACTGGACCTCTCGATAGAACACTCTACAAAGTACGAGTCTCTCTCCCTTCCAG 1860  
1861 GTGCATCTCCACACACACAGCACTAAGTGTCAATGCATTTTCTTTAAGGAAAGAAGAAT 1920  
1921 CTTTTTTTCTAGAGTCAACTTTCACTCACTCTAGCACAGCGGAGTGACTGCTGCATC 1980  
1981 TAAAAGGCAGCCAAACAGTATTCATTTTTTAATCTAAATTTCAAATCACTGTCTGCCT 2040  
2041 TTATCACATGGCAATTTTGTGGTAAAATAATGGAAATGACTGGTCTATCAATATTGTAT 2100  
2101 AAAAGACTCTGAAACAATTACATTATATAATATGTATACAATATTGTTTTGTAATAAG 2160  
2161 TGTCTCCTTTTATATTTACTTTGGTATATTTTACACTAATGAAATTTCAAATCATTAAA 2220  
2221 GTACAAAGACATGTCATGTATCACAAAAAGGTGACTGCTTCTATTTCAAGTGAATTAG 2280  
2281 CAGATTCAATAGTGGTCTTAAACTCTGTATGTTAAGATTAGAAGTTATATTACAATCA 2340  
2341 ATTTATGATTTTTTACATTATCAACTTATGGTTTCATGGTGGCTGTATCTATGAATGTG 2400  
2401 GCTCCAGTCAAATTTCAATGCCCCACCATTTAAAAATTACAAGCATTACTAAACATAC 2460  
2461 CAACATGTATCTAAAGAAATACAAATATGGTATCTCAATAACAGCTACTTTTTTATTTA 2520  
2521 TAATTTGACAATGAATACATTTCTTTTATTTACTTCAGTTTTATAAATTGGAACCTTGT 2580  
2581 TATCAAATGTATTGTAAGTCAAGTAAATGAAATTTTCTTACATAAAAATGTGTAGAA 2640  
2641 ACTATAAATTAAGTGTTCACATTTTGAAGGC 2676

## FIG.5b

1 AAGAAAAGTAAAGGAAGAAACAAGAACAAGAAAAAGATTATATTGATTTTAAAATCAT 60  
M  
61 GCAAAAACGCAACTCTGTGTTTATTTACCTGTTTATGCTGATTGTTGCTGGTCCAGT 120  
Q K L Q L C V Y I Y L F M L I V A G P V  
121 GGATCTAAATGAGAACAGTGTAGCAAAAAGAAAATGTGAAAAAGAGGGGCTGTGTAATGC 180  
D L N E N S E Q K E N V E K E G L C N A  
181 ATGTACTTGGAGACAAAACACTAAATCTTCAAGAATAGAAGCCATTAAGATACAAATCCT 240  
C T W R Q N T K S S R I E A I K I Q I L  
241 CAGTAAACTTCGTCTGGAAACAGCTCCTAACATCAGCAAAGATGTTATAAGACAACCTTT 300  
S K L R L E T A P **N I S** K D V I R Q L L  
301 ACCCAAAGCTCCTCCACTCCGGAACTGATTGATCAGTATGATGTCCAGAGGGATGACAG 360  
P K A P P L R E L I D Q Y D V Q R D D S  
361 CACCGATGGCTCTTTGGAAGATGACGATTATCACGCTACAACGGAAACAATCATTACCAT 420  
S D G S L E D D D Y H A T T E T I I T M  
421 GCCTACAGAGTCTGATTTTCTAATGCAAGTGGATGAAAACCCAAATGTTGCTTCTTTAA 480  
P T E S D F L M Q V D G K P K C C F F K  
481 ATTTAGCTCTAAAATACAATAAATAAAGTAGTAAAGGCCCAACTATGGATATATTGAG 540  
F S S K I Q Y N K V V K A Q L W I Y L R  
541 ACCCGTCCGAGACTCCTACAACAGTGTGTTGCAAATCCTGAGACTCATCAAACCTATGAA 600  
P V E T P T T V F V Q I L R L I K P M K  
601 AGACGGTACAAGGTATACTGGAATCCGATCTCTGAACTTGACATGAACCCAGGCACTGG 660  
D G T R Y T G I R S L K L D M N P G T G  
661 TATTTGGCAGAGCATTGATGTGAAGACAGTGTGCAAAAATGGCTCAAACAACCTGAATC 720  
I W Q S I D V K T V L Q N W L K Q P E S  
721 CAACTTAGGCATTGAAAATAAAGCTTTAGATGAGAATGGTCATGATCTTGCTGTAACTT 780  
N L G I E I K A L D E N G H D L A V T F  
781 CCCAGGACCAGGAGAAGATGGGCTGAATCCGTTTTAGAGGTCAAGGTAACAGACACACC 840  
P G P G E D G L N P F L E V K V T D T P  
841 AAAAAGATCCAGAAGGGATTTTGGTCTTGACTGTGATGAGCACTCAACAGAATCAGGATG 900  
K **R S R R** D F G L D C D E H S T E S R C  
901 CTGTGCTTACCCTCTAACTGTGGATTTTGAAGCTTTTGGATGGGATTGGATTATCGCTCC 960  
C R Y P L T V D F E A F G W D W I I A P  
961 TAAAAGATATAAGCCAATTACTGCTCTGGAGAGTGTGAATTTGTATTTTACAAAATA 1020  
K R Y K A N Y C S G E C E F V F L Q K Y  
1021 TCCTCATACTCATCTGGTACACCAAGCAAACCCAGAGGTTCAGCAGGCCCTTGTGTAC 1080  
P H T H L V H Q A N P R G S A G P C C T  
1081 TCCCACAAAGATGTCTCCAATTAATGCTATATTTTAAATGGCAAAGAACAATAATATA 1140  
P T K M S P I N M L Y F N G K E Q I I Y  
1141 TGGGAAAATCCAGCGATGGTAGTAGCCGCTGTGGGTGCTCATGAGATTTATATTAACC 1200  
G K I P A M V V D R C G C S \*

FIG.5c

1201 GTTCATAACTTCCTAAAACATGGAAGGTTTTCCCCTCAACAATTTTGAAGCTGTGAAATT 1260  
 1261 AAGTACCACAGGCTATAGGCCTAGAGTATGCTACAGTCACTTAAGCATAAGCTACAGTAT 1320  
 1321 GTAAACTAAAAGGGGAATATATGCAATGGTTGGCATTAAACCATCCAAACAAATCATA 1380  
 1381 AAGAAAGTTTTATGATTTCCAGAGTTTTGAGCTAGAAGGAGATCAAATTACATTTATGT 1440  
 1441 TCCTATATATTACAACATCGGCGAGGAAATGAAAGCGATTCTCCTTGAGTTCTGATGAAT 1500  
 1501 TAAAGGAGTATGCTTTAAAGTCTATTTCTTTAAAGTTTTGTTTAAATTTACAGAAAAAT 1560  
 1561 CCACATACAGTATTGGTAAAATGCAGGATTGTTATATACCATCATTCCAATCATCCTTAA 1620  
 1621 ACACTGAATTTATATTGTATGGTAGTATACTGGTAAGATAAAATCCACAAAAATAGG 1680  
 1681 GATGGTGCAGCATATGCAATTTCCATTCCTATTATAATGACACAGTACATTAACAATCC 1740  
 1741 ATGCCAACGGTGCTAATACGATAGGCTGAATGTCTGAGGCTACCAGGTTTATCACATAAA 1800  
 1801 AAACATTCAGTAAAATAGTAAGTTTCTTTCTTTTCAGGTGCATTTTCTACACCTCCAA 1860  
 1861 ATGAGGAATGGATTTCTTTAATGTAAGAAGAATCATTTTCTAGAGGTTGGCTTTCAAT 1920  
 1921 TCTGTAGCATACTTGGAGAACTGCATTATCTTAAAAGGCAGTCAAATGGTGTTTGTTTT 1980  
 1981 TATCAAAATGTCAAATAACATACTTGGAGAAGTATGTAATTTTGTCTTTGGAAAATTAC 2040  
 2041 AACACTGCCTTTGCAACACTGCAGTTTTTATGGTAAAATAATAGAAATGATCGACTCTAT 2100  
 2101 CAATATTGTATAAAAAGACTGAAACAATGCATTTATATAATATGTATACAATATTGTTTT 2160  
 2161 GTAAATAAGTGTCTCCTTTTTTATTTACTTTGGTATATTTTACACTAAGGACATTTCAA 2220  
 2221 ATTAAGTACTAAGGCACAAAGACATGTCATGCATCACAGAAAAGCAACTACTTATATTT 2280  
 2281 AGAGCAAATTAGCAGATTAATAGTGGTCTTAAAACCTCATATGTTAATGATTAGATGGT 2340  
 2341 TATATTACAATCATTTTATTTTTTTTACATGATTAACATTCACTTATGGATTCATGATG 2400  
 2401 GCTGTATAAAGTGAATTTGAAATTTCAATGGTTTACTGTCATTGTGTTTAAATCTCAACG 2460  
 2461 TTCCATTTTTTAATACTTGCAAAAACACTAAGTATACCAAAATAATTGACTCTATT 2520  
 2521 ATCTGAAATGAAGAATAAACTGATGCTATCTCAACAATAACTGTTACTTTTTATTTATAA 2580  
 2581 TTTGATAATGAATATATTTCTGCATTTATTTACTTCTGTTTTGTAATTTGGGATTTTGT 2640  
 2641 AATCAAATTTATGTACTATGACTAAATGAAATTTTCTTACATCTAATTTGTAGAAAC 2700  
 2701 AGTATAAGTTATATTAAGTGTTTTTACATTTTTTTGAAAGAC 2743

FIG.5d

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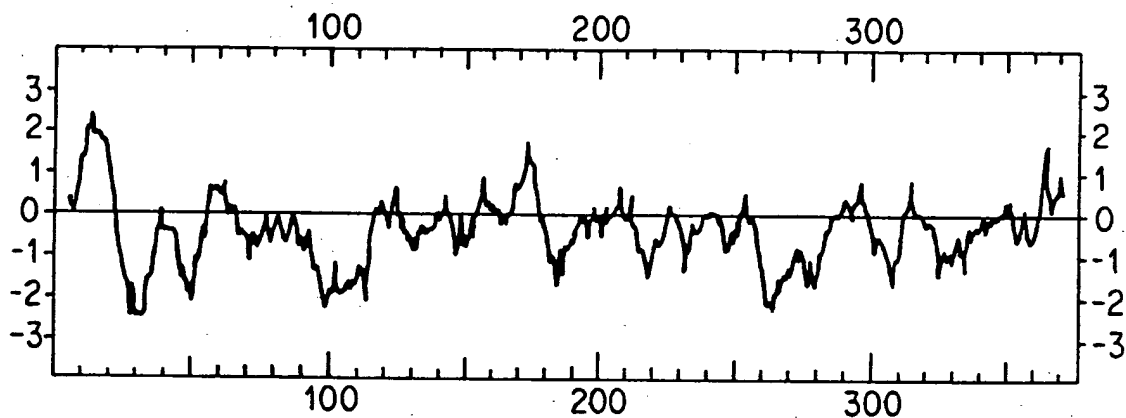


FIG. 6a

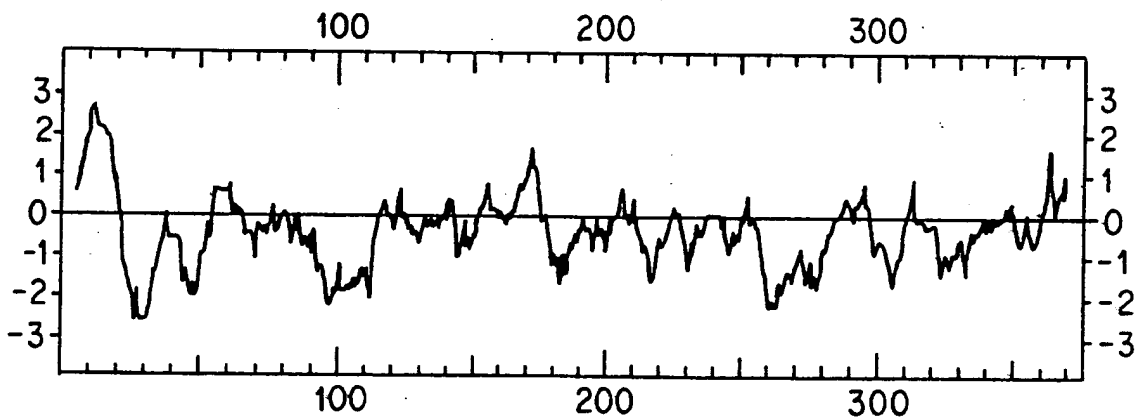


FIG. 6b



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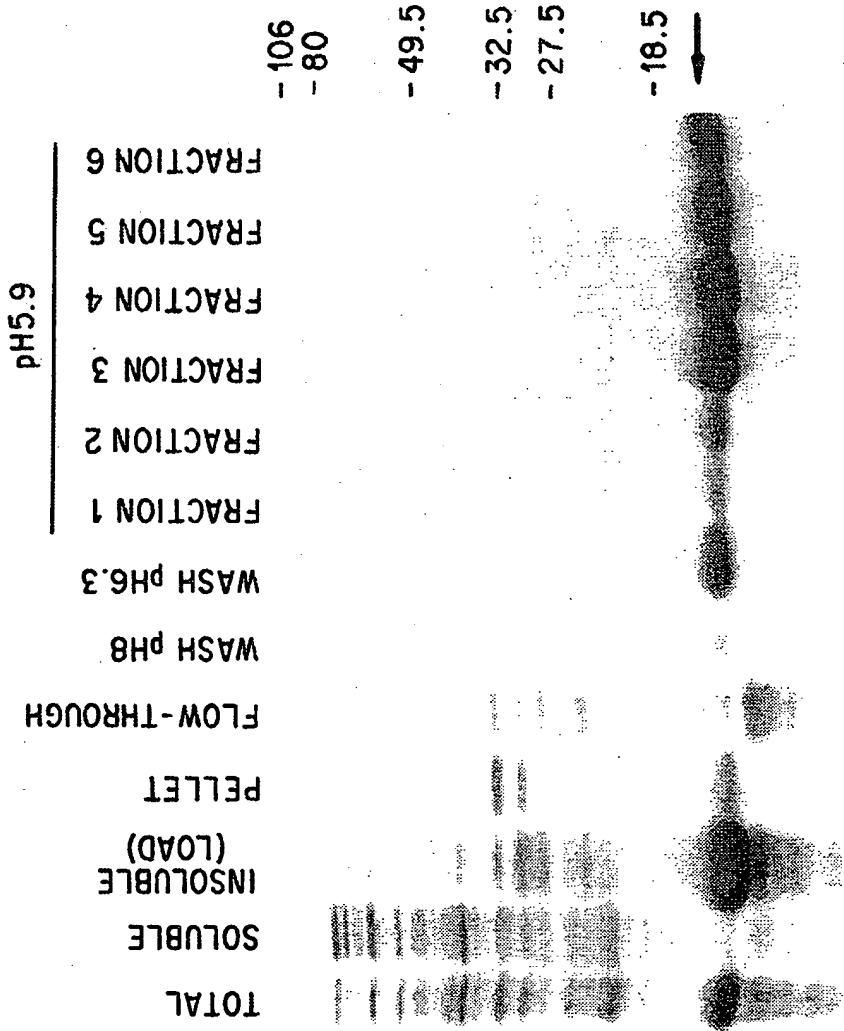


FIG. 8

12/15

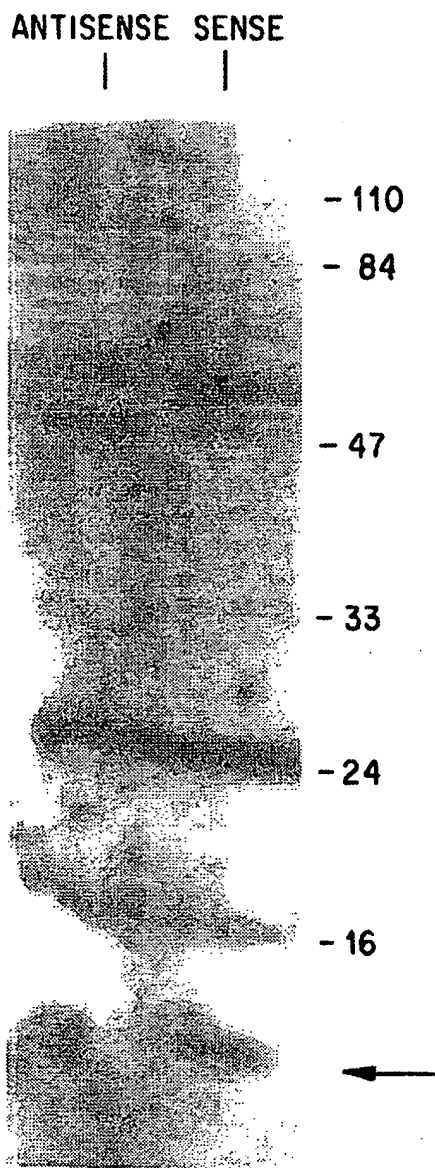


FIG. 9

-2.9 kb

UTERUS  
 FAT  
 OVARY  
 LIVER  
 MUSCLE  
 TESTIS  
 SPLEEN  
 INTESTINE  
 PANCREAS  
 SEMINAL VESICLE  
 KIDNEY  
 BRAIN  
 THYMUS  
 LUNG  
 HEART



FIG. 10a



14/15

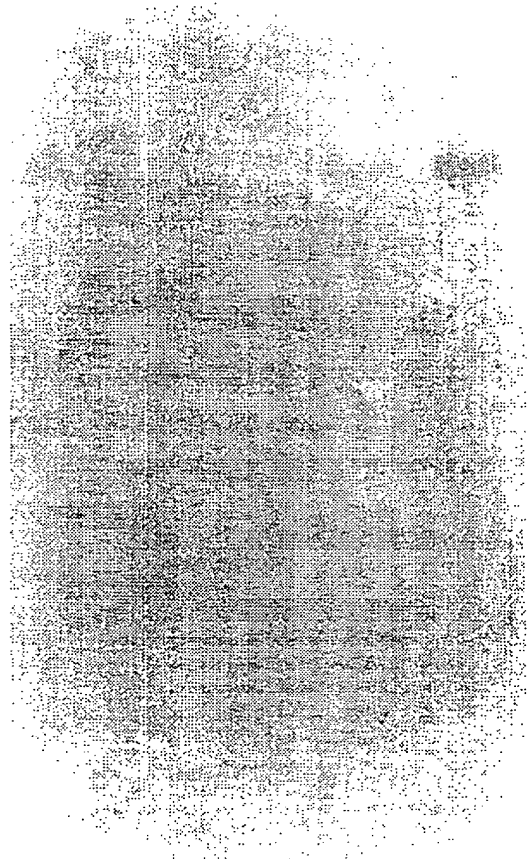
12.5 d PLACENTA

14.5 d PLACENTA

16.5 d PLACENTA

12.5 d EMBRYO

18.5 d EMBRYO



- 2.9 kb

**FIG. 10b**

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 X Y M | H B1  
CHO

1018 —  
506/517  
396  
344  
298



FIG. 11

**INTERNATIONAL SEARCH REPORT**

International application No.  
PCT/US94/03019

**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : C07K 13/00, 15/28; A61K 37/36; C12N 15/18, 15/10, 15/66  
US CL : 530/399, 387.1; 536/23.5; 514/12; 435/69.1, 320.1, 252.3

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/399, 387.1; 536/23.5; 514/12; 435/69.1, 320.1, 252.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Molecular Endocrinology, VOLUME 6, NUMBER 11, issued 1992, "Isolation of Vgr-2, a Novel Member of the Transforming Growth Factor-beta-Related Gene Family", pages 1961-1968.	1-43
A	Proceedings of the National Academy of Sciences, VOLUME 88, issued May 1991, "Expression of growth/differentiation factor 1 in the nervous system: Conservation of a bicistronic structure", pages 4250-4254.	1-43
A	Molecular Endocrinology, VOLUME 4, NUMBER 7, issued 1990, "Identification of a Novel Member (GDF-1) of the Transforming Growth Factor-beta Superfamily", pages 1034-1040.	1-43

Further documents are listed in the continuation of Box C.  See patent family annex.

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*O* document referring to an oral disclosure, use, exhibition or other means	
*P* document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 29 APRIL 1994	Date of mailing of the international search report <b>18 MAY 1994</b>
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Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Shelly Guest Cermak <i>Jill Warden for</i> Telephone No. (703) 308-0196
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# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US94/03019

## C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	The Journal of Biological Chemistry, VOLUME 268, NUMBER 5, issued 15 February 1993, "GDF-3 and GDF-9: Two New Members of the Transforming Growth Factor-beta Superfamily Containing a Novel Pattern of Cysteines", pages 3444-3449.	1-43

**B. FIELDS SEARCHED**

Electronic data bases consulted (Name of data base and where practicable terms used):

APS, Dialog: File Biochem. Medicine  
search terms: Growth differentiation factor-8, GDF-8  
Sequence Data: PIR, SwissPro, GenBank

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